IRON BIOAVAILABILITY IN INDIA:

HISTORICAL PERSPECTIVES AND CURRENT CONCERNS

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IRON BIOAVAILABILITY IN INDIA: HISTORICAL PERSPECTIVES AND CURRENT CONCERNS

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Indian women and children continue to suffer the highest rates of anemia in the world the despite economic and agricultural growth of the past four decades. High rates of iron deficiency anemia are attributed to low iron intakes and, perhaps more importantly, to low iron bioavailability from diets high in cereals and low in animal source foods. A better understanding of trends in iron deficiency risk in India over the past thirty is warranted and examined through intakes of dietary bioavailable iron. Adult 24-hour recall data from four cross-sectional survey rounds in 1975-80, 1996-97, 2000-01 and 2004-05 (n=45,026) were analyzed. A bioavailability algorithm was used to calculate dietary bioavailable iron (DBI) for each individual based on iron intake as well as intake of major iron inhibitors and enhancers, like phytates, tannins and ascorbic acid. Objectives of the research were to understand trends in DBI, to compare cereal-based diets in their ability to provide DBI and finally, the potential impact of iron-biofortified crops on improving DBI intakes. Results indicate that unlike iron intakes, which have remained unaltered from 1975-2000, DBI has improved, due to dietary shifts increasing iron bioavailability. However, trends indicate a drop in DBI in the last five years and parallel recent anemia findings. Analysis of specific cereal-based diets reveal that pearl millet and wheat diets are more protective against low DBI intake (<50% of basal requirements for iron) than rice based diets. Finally, iron biofortified rice and wheat have the potential to increase

DBI intake levels to shift at least 4.5 million people out of iron deficiency. Findings indicate that the risk of iron deficiency has reduced over the past thirty years, with the exception of the last five years, and research on improving pearl millet production and/or continuing research on iron-biofortified rice could significantly reduce iron deficiency in India. Finally, this research highlights the need to examine iron intake at the level of bioavailable iron and that bioavailability algorithms, though they may require further refinement, are a useful tool for iron nutrition.

BIOGRAPHICAL SKETCH

Born the daughter of a diplomat, Christina spent her childhood outside her native United States, in Guatemala, Brazil, France, Greece and Spain. She obtained her Bachelor of Science at the College of William & Mary in 1994 as a dual major in Biology and Anthropology. After spending two years as a Peace Corps health extension in rural Nicaragua she returned to the United States to study public health, completing her Master's in Public Health from Emory University Rollins School of Public Health with a concentration in International Health and Nutrition in 2001. Better trained and prepared to contribute to the field of international nutrition, she worked as a research specialist for Harvard School of Public Health based in Dar es Salaam, Tanzania working with colleagues on clinical trials focused on examining the impact of multivitamins on pregnancy outcomes among both HIV-positive and HIV-negative Tanzanian women. After over two years of this field research, Christina was inspired her to return to academia in 2004 to work towards a doctorate in International Nutrition at Cornell University.

Christina's studies at Cornell included not only nutrition, but also coursework for her minors in both Agricultural Development and Applied Economics. She conducted her doctoral fieldwork in Hyderabad, India working with the National Institute of Nutrition and the International Crop Research Institute of the Semi-Arid Tropics.

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On a personal note, I could not have completed this without the love and support of my husband, Ibadat Dhillon, who stood by me through the blood, sweat and tears that, without exaggeration, represent this work.

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CHAPTER 1

INTRODUCTION

Iron deficiency anemia is the most prevalent micronutrient deficiency in the world affecting roughly 2 billion people [1]. In absolute numbers India is home to one third of the worlds' anemic population, with over 70% of women and children affected [2]. The unusually high prevalence of anemia in India is attributed to diets low in both iron and iron bioavailability. Most rural Indians rely heavily on basic cereal grains for the bulk of their calories and therefore for their iron intake, as consumption of animal-source foods is not widespread. These dietary characteristics also contribute to low iron bioavailability. In fact, recommended intakes for iron in India are set higher than for most countries because of the low iron bioavailability- so little of the iron consumed in Indian diets is available for absorption.

Common cereals in India include rice, wheat, sorghum and millets. India's agricultural green revolution tripled the total production of rice and wheat, increasing the *per capita* availability of both while production of sorghum, millets and pulse crops lagged behind population growth. Among the major grains consumed in India, these coarse cereals and pulses, have four to ten times as much iron as rice, but also substantially more iron-inhibiting compounds which lower iron bioavailability. In the context of iron nutrition, it is not well examined if either a high iron/low bioavailable coarse cereal diet or a low iron/high bioavailable rice diet (given the accompanying diet) provide a better source of iron for the average Indian. We do know that historically the prevalence of iron deficiency anemia in India among women and young children has not shown much decline since the 1960s [1-12] and most recently, has increased [13, 14].

This body of work provides three papers that retrospectively examine the Indian diet and its provision of dietary bioavailable iron (DBI). Secondary analysis is conducted from a large national dataset from the National Nutrition Monitoring Bureau (NNMB), which has collected four rounds of dietary data spanning thirty years, in rural areas of seven Indian states (Kerala, Tamil Nadu, Maharashtra, Karnataka, Andhra Pradesh, Gujarat and Orissa). The first paper is both descriptive, outlining thirty-year trends in Indian DBI intakes, and methodologically innovative, introducing the application of an iron bioavailablity algorithm to retrospective data as a tool for dietary analysis. The second paper builds on this work to further examine Indian diets by cereal-base, specifically their ability to provide for DBI among rural consumers in the context of the whole diet. Analysis of diet types is essential, given the differing iron bioavailability and iron content of the major Indian cereals, and will help establish dietary modifications which can increase DBI given through their enhancing compounds and/or the iron they offer. Such analyses would be valuable in determining dietary recommendations for the most vulnerable population for iron deficiency. Finally, the third paper uses the most current dietary intake data to predict the impact iron-biofortified rice and wheat would have in improving DBI in this rural Indian population. Such 'biofortified' crops, currently being bred by agricultural research centers worldwide, hold great promise in delivering more iron in cereal-based diets, but has never been quantitatively examined for its potential impact at the level of iron bioavailability. Examination of the amount of *bioavailable* biofortified iron in these crops will provide a more realistic estimate of absorbed iron and therefore a better estimate for the crops' potential impact on combating iron deficiency.

This closer examination of not just iron but bioavailable iron is needed in India in order to shed more light on the high prevalence of iron deficiency seen. The application of an iron bioavailability algorithm is the only way to estimate iron bioavailability in large and/or retrospective datasets. Use of these bioavailability algorithms has been limited thus far and their application to dietary data is a unique contribution of this body of work to the literature. The author hopes to inform the nutrition community of their broader use, expanding the methodological toolbox for researchers interested in studying dietary iron deficiency in resource poor settings.

Empirical evidence will provide us with much needed historical trends on dietary iron deficiency in India, and will help quantify the impact of dietary choices on iron deficiency risk in India. Results will provide evidence for both the formulation of food policy in India, including the potential impact of biofortified crops on bioavailable iron intakes. Finally, results may provide nutritional 'lessons learned' from South Asia's agricultural revolution for Africa, where a green revolution is just underway.

CHAPTER 2

IRON BIOAVAILABILITY IN INDIA: HISTORICAL PERSPECTIVES

Introduction

Iron deficiency anemia continues to be a significant public health problem in India. Low iron bioavailability has been attributed to the high consumption of cereals and low consumption of animal source foods. Cereals provide the bulk of both iron and iron absorption inhibitors like phytates and tannins. It is unknown how dietary iron deficiency, considering iron absorption, has changed over time in India. Historical perspectives on anemia trends, which serve as the best proxy for overall iron deficiency, are limited and inconclusive. This research attempts to determine recent trends in iron intake, iron bioavailability and dietary iron deficiency in India since the 1970s. Such information may help shed some light on the persistence of iron deficiency anemia despite the country's improvements in caloric intakes and the concurrent economic growth.

Background

Iron deficiency anemia in India

Prevalence of iron deficiency anemia (IDA) in India is consistently the highest in the world. It is generally agreed that over 70% of women and children are anemic [1, 2, 5, 7, 10], making India host to the largest population of anemic individuals in the world. The most recent Demographic Health Survey estimates for 2005 anemia prevalence among ever-married women, children, pregnant women and men are 56.2%, 79.2%, 57.9%, and 24.2% respectively [14]. Clinically, anemia is the inability of the body to produce sufficient hemoglobin for the optimal transport of oxygen throughout the body. It principally affects pregnant women and children due to their physiological increases in blood volume from reproduction and growth. Although anemia can be caused by infections (e.g. malaria, hookworm), hemoglobinopathies (e.g. sickle-cell, thalessemia), and nutrient deficiencies, like iron, folate, vitamins A, C or B12, the predominant cause of anemia worldwide is due to a deficiency in iron [1].

The rates of anemia reflect just the tip of the iceberg of the prevalence of iron deficiency in a population. Population prevalence estimates for iron deficiency are generally accepted to be two and a half times the prevalence found for iron deficiency anemia within that population, implying that in India nearly every woman and child and roughly half of all adult men are iron deficient [1, 14]. This high prevalence of iron deficiency in India has been attributed to both low iron intakes and low iron bioavailability from diets with high levels of cereal consumption and low intakes of animal source foods [15].

Iron deficiency can be caused by a dietary deficiency in iron and/or excessive iron loss, usually via blood loss. This blood loss can be due to parasitic infections such as hookworm or schistosomiasis, or from hemorrhaging due to childbirth or heavy menstruation. Anemia not caused by iron deficiency represents a small portion of the total anemia in the world and can be caused by hereditary disorders (e.g. thalessemias, glucose-6-phosphate dehydrogenase deficiency), malaria-associated hemolysis, or a deficiency in vitamin A, B-12, C or folic acid. Because hemoglobin concentration is a field-friendly test, data for anemia prevalence is usually more frequently available than data for iron deficiency or for IDA specifically, where secondary and more expensive tests like serum ferritin concentration, mean cell volume, or transferrin saturation would also be required. For this reason, anemia is often used as a proxy for estimating the prevalence of iron deficiency in a population. In India there is much reason to believe that most of the anemia is caused by iron given the dietary limitations discussed (low iron diet, low iron bioavailability), though rural areas of India also exhibit high rates of parasitic infection, and pockets of thalessemia cases exist in tribal areas. Based on regional studies in India, the percent of anemia attributed to a deficiency in iron is somewhere between 65%-90% of the anemia cases [10, 16]. Although dietary iron deficiency is a significant cause of the iron deficiency give the low bioavailability and high vegetarian diets common in India, the high rates of anemia seen among men may indicate that hookworm infection is also a large contributing factor [17]. **Figure 2.1** illustrates this relationship. Of concern in this research is chronic dietary iron deficiency in the population and therefore anemia is discussed as a proxy for the more widespread problem of iron deficiency.

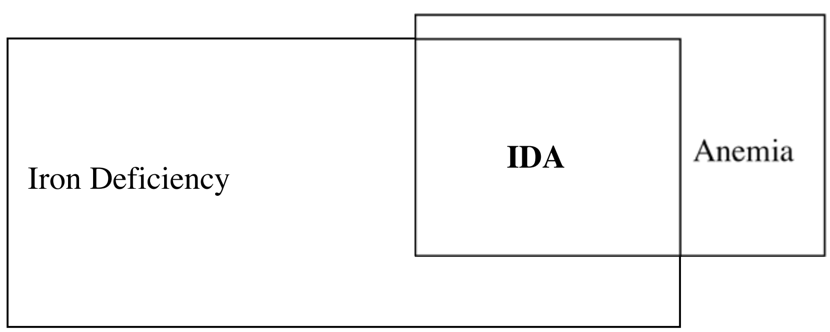

Figure 2.1. Relationship between iron deficiency and anemia
Source: UNICEF 2002 [1, 12], adapted from Yip 1989 [18]

On a spectrum of iron status, iron-deficiency anemia (IDA) sits at one extreme as the most severe form of iron deficiency, followed by iron deficiency without anemia, iron depletion, normal status and finally iron overload at the other end of the spectrum. IDA is defined by the World Health Organization as hemoglobin concentrations levels <11 g/dL for pregnant women, <11.5 g/dL for children 5-11 years of age, <12 g/dL for adult women and children 12-15 years old and <13 g/dL for adult men (15 years or older) [1]. Iron deficiency without anemia is defined as no anemia but serum ferritin <12 μg/l for all age groups according to the definition of the Indian Council of Medical Research (<15 μg/l according to the WHO)[1, 19].

The evidence indicates that IDA causes the most severe of these consequences to individuals, but non-anemic iron deficiency can also take its toll by lowering physical work capacity [20-24], increasing risk of maternal mortality [1, 12] and among children, impairing cognitive development [25-32]. These consequences come from reduced activity of iron-dependent enzymes as well as the diminished capacity of the body to transport oxygen [33]. Although iron therapy can improve some of these outcomes, the cognitive impairments sustained in early childhood have been shown to be irreversible and therefore cause a lifetime of lost potential [26, 27, 32]. This endemicity of iron deficiency in India accounts for roughly 22,000 maternal deaths [7] and an estimated $3.8 billion in lost productivity each year [34].

Trends in anemia prevalence

Historical data on iron deficiency anemia in India can be difficult to piece together as there is no single set of prevalence estimates that has been consistently measured across time. Methodologies for determining anemia have varied and sample populations have not always been representative to the greater population. For South Asia in general, successive ACC/SCN Reports on the World Nutrition Situation indicate an increasing prevalence of anemia among non-pregnant adult women 15 and 49 years of age in the region since 1972 (57%, 67%, 72%, and 75% for 1972, 1981-1984, 1985, and 2000 respectively) [3, 4]. More specific to India, various trend analyses have been attempted for India. Some report increased prevalence among non-pregnant women from the 1970s to the mid 1980s with a decline since, ranging from 55% to 70% and back down to 51% [2]. A recent meta-analysis in India concludes that anemia prevalence in India among pregnant women has increased from 80.7% during the period of 1950-90 and 83.6% since 1991 [11]. Their prevalence estimates for infants and children were 77% pre-1970, 72% between 1971-1990 and remained 70% since 1991, showing a general decline. Another study claims from 1990 to 2000 prevalence among women has not changed, but among preschool age children it has declined from 80% to 75% [7]. Given the large time periods used for point estimates (forty years in some cases), the lack of a consistent trend across different subpopulations, and the inability to specify regions of India where data were collected (as no national data were used in any of these analyses) it is hard to conclude anything about the historical pattern of iron deficiency in India, except to say that anemia rates are and have remained high by any global standard. Trends in dietary bioavailable iron would help to better understand the patterns of iron deficiency exhibited in India over the last thirty years.

The most reliable estimates for anemia are based on two repeated surveys from the Demographic and Health Survey (DHS) in India, but have only been collected since the late 1990s. Analyses from 1998-99 and again in 2004-05 using the same sampling frame and methodology indicate a rise in anemia prevalence in at least one of the three repeat sampled populations (pregnant women, ever-married 15-49 year old women, and 6-35 month old children) in all of the 28 states of India, except Jammu in the far north [13, 14]. Prevalence estimates for 2005 anemia prevalence among ever-married women, children, pregnant women and men are 56.2%, 79.2%, 57.9%, and 24.2% respectively [14]. Some in the nutrition community in India claim the DHS estimates are too low, citing that the gold standard cyanmethemoglobin method was not used, but rather the Hemocue methodology [5, 8]. Regardless, relative interstate differences were consistent and therefore the increasing trend in anemia prevalence has not been refuted. The Indian states of Kerala, Gujarat, Andhra Pradesh, Karnataka, Madhya Pradesh and Uttar Pradesh show increased anemia prevalence in all three sub-populations over the past 6 years. These reports have generated substantial political concern in India about the country's failure to reduce anemia prevalence [35, 36].

Unsuccessful anemia prevention program

Iron folate tablets have been distributed for 30 years in India through the primary health care system to pregnant and lactating women as well as children under the age of five. Yet despite the global success in treating and preventing anemia through iron supplementation, improvements in India have not been seen. Evaluations of the Indian program state that iron supplementation interventions have had no biological effect on the targeted population, and claim that of the targeted population less than 20% of women and less than 1% of children were reported to have even been given the supplements [37, 38]. Explanations for its inadequate coverage include poor infrastructure, inefficient distribution systems and insufficient funds. According to reports, the government contributes only 10% of the funds required for all three of their micronutrient programs combined (vitamin A, iron and salt iodization) [37, 38]. Fortification programs have been considered ‘negligible’ in contributing to iron intake [35] and are primarily aimed at the urban poor. Due to the prohibitive cost of supplementation and fortification programs in a country as large and diverse as India, micronutrient intakes are still highly dependent on basic food crops, and will remain that way for a long time to come for the most inaccessible rural populations.

India’s diversity

India represents the second most populated nation in the world with roughly 1.1 billion people, all of whom live in a land area just over one-third of that of the United States. The country is composed of 28 states that are divided into 6 major regions (see map Appendix A). Kerala stands apart from the rest of India as having health indicators (including infant mortality, maternal mortality, female education) far better than the national average and often as good as developed countries [39]. India is as diverse as its terrain, representing a broad spectrum of agricultural zones, diets, religions, and languages. It is hard to generalize about a country that has a population larger than the continent of Africa, where each individual state of India is comparable to an individual country not only in population size, but also in the variation of culture, food and language. Dietary differences from one state to another also vary greatly. In general, rice is consumed throughout India and grown in the southern and eastern states. Wheat, the second most consumed crop in India is grown and consumed mostly in the northern states. Coarse or traditional cereals are consumed mostly as a subsistence crop in pockets throughout the southern and central regions of the country. Although wide variations exist in dietary patterns across India, 60-70% of daily energy supplies come from cereals, regardless of socioeconomic status [40].

Dietary change in India

The past 40 years have seen significant consumption pattern changes in India due to many changes in the economy. In the 1970s-1980s, the agricultural 'green revolution' tripled rice and wheat production, which led to income growth in the 1980s and finally, the 1990s ushered in economic reforms which liberalization Indian economy.

An examination of consumption changes from 1972 to 1994 by the Nutrition Foundation of India indicates that although average consumption of cereals dropped in the rural population, among the lower income groups cereal consumption increased, predominantly from rice and wheat [41]. They attribute the increase to the need among the poorest to make up for caloric insufficiencies. India's Public Distribution System (PDS), a social welfare program which offers subsidized wheat, rice, oil and sugar to qualifying families ensured the benefits of agricultural growth were accessible to all. Consistent with the "Engel curve", the study shows that as incomes rose from 1972 to 2000, the percent of monthly per capita expenditures on food items declined among rural households from 70.6% to 55.3%. Expenditures for pulses and vegetables, however, increased among the poorest consumers due to their higher relative price. Therefore, as consumers, the poor in India were more and more able to afford basic calories but not as able to afford to diversify their diets. The study concludes that 'dietary diversity' (though it is not clear how this is measured) in 2000 was only seen among middle and high income groups [41].

Although the agriculture sector only represents one quarter of the country's gross domestic product, it generates income for roughly 60% of the population most of whom are rural farming families [42]. Growth in the agriculture sector impacts rural households through both lower food prices and higher incomes. Among the rural population, income benefits from agricultural growth were concentrated in areas where irrigation, suitable agricultural climates and green revolution technologies allowed for greater productivity [43-45]. States considered beneficiaries of this growth were initially those of the Indo-Gangetic Plain from Punjab to Uttar Pradesh, and in later years, the Eastern and Southern states as well, like Orissa, Andhra Pradesh and Tamil Nadu.

The net effect of higher incomes and cheaper cereals in the rural population was that energy intakes among the poor increased, which may help explain the modest decline in percent underweight seen from 1978-1989 (prevalence of preschool underweight dropped from 71% to 63%) [46, 47]. In **Figure 2.2**, per capita food consumption trends for all of India, rural and urban, based on FAO balance sheets of available food supplies from 1961-2001 are displayed. These trends reflect well the rise in per capita production of rice and wheat. In addition, consumption of vegetables and fruits increased over this period. Declines in mean consumption among coarse cereals and pulses were seen, however.

Dietary data from rural India on repeated household surveys from 1972 to 1996 reflect some of the macro-level consumption patterns seen in the FAO chart. According to the National Institute of Nutrition, during this time rural consumers show a decline in

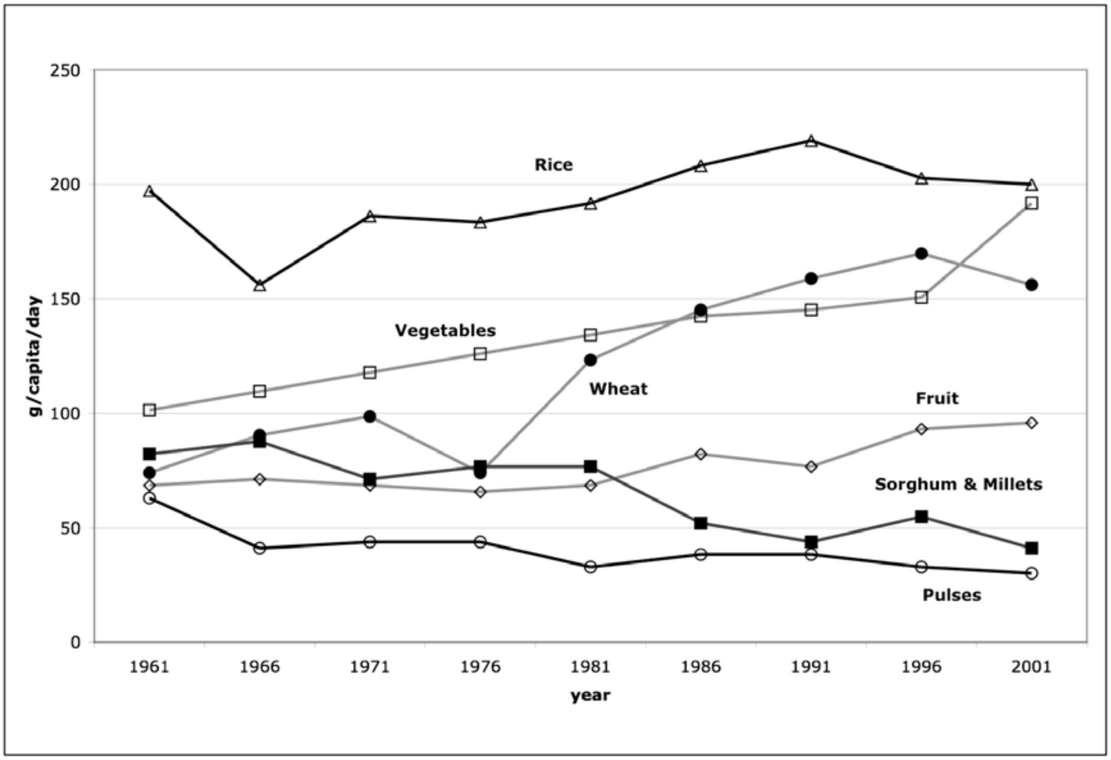

Figure 2.2. Per capita food consumption in India from 1961-2001
Based on FAO balance sheets where consumption = production + imports – exports – waste (including livestock consumption), source: FAOSTAT 2008 [48],

the consumption of cereals and dairy, an increase in the consumption of green leafy vegetables and little change in the consumption of pulses, fats and sugars. Resulting nutrient intakes for protein, energy, calcium and iron (iron values adjusted to revised iron content values using adjustment factor published in Toteja & Singh 2004 [11]) declined, while there was an increase in intake of vitamin A. However, it should be noted that these results reflect pooled calculations and therefore have not been adjusted for sampling or population weights [49] and so do not reflect true estimates for the population examined. Additionally, ranges and p-values for significant differences across the two time periods were not provided.

Table 2.1. Dietary and nutrient intakes in rural India from 1975 to 1997

	Unit	1975-80	1996-97
Cereals	g/CU[1]/day	505	450
Pulses	g/CU[1]/day	34	27
Green Leafy Vegetables	g/CU[1]/day	8	15
Other Vegetables	g/CU[1]/day	54	47
Dairy	g/CU[1]/day	116	86
Fats	g/CU[1]/day	14	12
Sugars	g/CU[1]/day	23	21
Protein	g/CU[1]/day	61.5	53.7
Energy	kcal/CU/day	2349	2108
Calcium	mg/CU/day	606	521
Iron[2]	mg/CU/day	17.9	14.2
Vitamin C	mg/CU/day	39	40
Vitamin A	mcg/CU/day	246	300

Source: NNMB 1999 [49]

[1]CU= consumption unit, where adult male=1 CU based on energy requirements by age and sex

[2]Calculated for new iron content values using adjustment factor in Toteja & Singh [11]

Compared to the urban population, the rural diets comprise lower intakes in all food and nutrient categories per consumption unit except among cereals, green leafy vegetables (GLVs), calories and iron [40]. (Consumption Units are a standardized measure for individuals based on energy requirements, where an adult male=1 CU). Dietary diversity is generally lower among the rural poor whose diets are often described as monotonous and heavily dependent on cereal consumption.

More recent dietary changes due to the liberalization of the economy post 1991 in India have been examined by Mahendra et al in 2004 [37]. Although domestic rice prices in the pre-reform era were 61% less than world prices, they remained stable after reform (examined from 1995 – 1998). On the other hand domestic wheat prices increased more dramatically, including in Public Distribution Shops (a subsidized food welfare program) and in governmental price supports to protect producers. Results on the impact of India's economic growth of the 1990s on poverty reduction have been highly controverted, but there is general agreement that income inequality has increased and mild, if any, reductions in poverty were seen [50-52]. Since the year 2000 the price of cereals on the world market have been increasing steadily [53], likely hurting mostly the poor net-consumers in India. After a dramatic upturn in early 2008 in cereal prices, India declared an export restriction on rice in April 2008 [54] to ensure that sufficient rice stayed in the country. The impact on poor net-producers has not yet been examined.

Trends in iron nutrition

As seen above, iron intakes declined by about 20% from 1975 to 1996. Most dietary iron in India comes from cereals due to its heavy consumption in a predominantly vegetarian population [55]. Data from ICRISAT Village Level Survey on dietary intakes from rural Maharashtra and Andhra Pradesh indicate that 73% - 82% of iron comes from cereals[56]. The cereals most commonly consumed in India are rice, wheat, some maize and the coarse or traditional cereals of sorghum and millets. Coarse cereals like sorghum and millet are roughly four times higher in iron than rice, gram for gram, as seen in **Table 2.2**. Despite the iron content of traditional cereals, according to the National Nutrition Monitoring Bureau (NNMB) in rural areas in 1997 only 5.6% of household surveyed were consuming at or above the recommended intake for iron (28 mg/CU/day) [49]. Other important sources of iron in Indian diets include vegetables and legumes. On average, pulses and green leafy vegetables offer 7 times the amount of iron from rice (~5mg/100g vs. 0.7 mg/100g), though they tend to be consumed in smaller quantities. But iron intakes alone are not sufficient to

Table 2.2: Comparison of iron and iron inhibitor content in major Indian cereals

	Iron (mg/100g)	Phytate (mg/100g)	Tannin Equivalents (mg/100g)[1]	Calcium (mg/100g)
Rice (milled)	0.7	288	0	10
Wheat (whole, flour)	4.9	795	23	45
Pearl Millet	8.0	494	13	42
Finger Millet	3.9	732	360	344
Sorghum	4.1	602	77	25
Bengal gram	6.3	497	38	71
Pigeon Pea	2.7	595	50	73

source: NIN Nutritive Value of Indian Foods unless otherwise indicated
[1]tannin content data is from appendix in Hallberg & Hulthen 2001 [57]

determine risk of iron deficiency, for iron bioavailability from one diet to another can vary greatly.

Iron bioavailability & absorption

Anywhere between 1% and 25% of the iron consumed is able to be absorbed into the body for use [58]. Dietary iron must be made available to be absorbed, given different components of the diet and the general digestive mechanisms. Then the bioavailable amount is absorbed in a fraction dependent on the iron status, developmental status and gut microflora of the individual [59].

Dietary iron is consumed as either heme (found only in animal foods) or non-heme iron (found in both animal and plant foods). Non-heme iron is the predominant form of iron consumed and its bioavailability can range from 1% - 15% depending on diet composition and individual iron status, while heme iron is absorbed in a more narrow range around 25% [60], depending on an individual's iron status. The strongest enhancer of iron absorption is low iron status. This adaptive feedback system which increases uptake of iron in the gut as iron stores decrease allows the body to maintain levels of iron necessary for oxygen transport and iron-dependent enzymatic processes. Low iron status can enhance absorption of iron by as much as 15-fold [61] while diet composition, or the enhancers and inhibitors of iron in the diet, can alter absorption by at most a factor of ten.

Dietary inhibitors of iron include calcium, phytates, and polyphenols. Phytates, which bind with iron in the presence of calcium or magnesium to make iron unavailable for absorption in the small intestine, generally has the strongest dietary effect on iron bioavailability. Phytate concentrations are highest in seeds, legumes and unrefined cereals (whole grains). Polyphenolic compounds are also found to inhibit iron bioavailability in the gut. Teas have high amounts of phenolics, as well as darker skinned pulses, which get their color from phenolics called tannins. Tannins are also found in millets and many spices, including turmeric, a ubiquitous spice of India. Tannins bind to iron irreversibly, creating insoluble iron compounds unavailable for human absorption. Enhancers of iron bioavailability include ascorbic acid (vitamin C), which reduces ferrous ions to absorbable ferric ions, and meats/fish/poultry (MFP), often called the ‘meat factor’, whose enhancing mechanism is still not well understood. For India, a country with a high rate of vegetarians for both religious and economic reasons, non-heme iron is the predominant form of iron in the diet.

Compared to other cereals, rice offers less iron per gram but also has fewer iron inhibitors. Most other cereals in India are consumed unrefined and therefore their phytate and tannin content is relatively high, whereas with rice most of these inhibitors are lost during milling. For a breakdown of nutrient and anti-nutrient content of these cereals, see Appendix B. These opposing ‘strategies’ of either high iron/low bioavailability or low iron/high bioavailability have not been fully examined for their net effect on the amount of dietary iron that is bioavailable for individuals. This net affect on the amount of dietary bioavailable iron is dependent on the whole diet, in addition to the cereal itself.

It should be noted that in India milled rice is considered to hold 0.7 mg of iron per 100 grams of rice, a content value higher than most rice values in other parts of Asia and the world where 0.3 mg of iron per 100 grams of rice is more commonly seen [62, 63]. Food composition data for India were most recently updated in 1989, and were gathered from laboratory analyses conducted in various Indian universities and/or from published data from Indian foods using AOAC-approved analyses for mineral content [55]. In order to allow for comparability of our findings to the local context, all nutrient content data from the Indian food composition table are used, unless they are not available.

Iron bioavailability in the Indian diet

The bioavailability of iron in the Indian diet is very low and is often cited as a reason why iron deficiency is so prevalent in this country. Whereas in most developed countries roughly 18% of dietary iron is available for absorption, in India estimates are closer to 5% for the average individual. The Indian Council of Medical Research (ICMR) establishes dietary recommendations for daily iron intakes based on the factorial method, which calculates the expected daily iron losses of an individual (from cell sloughing, sweating, menstrual blood loss) as a function of physiological status, age and sex and adjusting for the iron bioavailability of the diet. The World

Health Organization estimates dietary iron requirements in the same way, except that it uses the same bioavailability estimate for all age and sex groups as shown in Table 3, and adjusts upwards to cover most of the variation found in the population, not just the 'average' individual in the population. Therefore, according to the ICMR, the average woman loses 1.5 mg of iron a day (therefore their basal requirement) and consumes a mixed-cereal diet, will need to consume 30 mg of iron on the basis that she only absorbs 5% of the iron she consumes, in order to recover basal loses. The WHO estimates similar basal losses and in addition to accounting for a low bioavailability of a standard 5%, adds to the requirement to ensure that 97.5% of the variation within the population is sufficiently covered (the mean + 2 standard deviations). Therefore according to WHO recommendations, an adult woman should consume roughly twice the amount of iron (58.8 mg) recommended by the ICMR [64]. Therefore the ICMR has developed their requirements as an estimated average requirement (EAR, or what half the healthy population would require) and the WHO as a recommended daily allowance (RDA, or enough to ensure that 97.5% of people would fulfill their requirements). Estimated average requirements are generally a better measure for population-based studies so that deficiencies are not overestimated, and RDAs are more suitable for individual recommendations to ensure adequacy for a given individual [60].

The ICMR estimates average iron bioavailability in the Indian context for three different types of diets: rice, wheat/millet or mixed cereal-based diets. This is because of their very different content of iron inhibitors. Their relative bioavailabilities are estimated at 5%, 2%, and 3%, respectively [19]. They adjust iron absorption based on an individual's physiological status (as a proxy for iron stores) since low iron status causes upregulation of iron absorption. For example, it is estimated that a pregnant woman (whose iron status is expected to be low) will absorb 13.3%, 8% and 5.3% of the iron in rice, wheat/millet and mixed cereal diets, respectively. These bioavailabilities were determined using extrinsically labeled iron and calculated from mean iron absorption in typical Indian meals (many of which are vegetarian). Estimates were developed for various sub-populations including children of different ages and gender, lactating women, pregnant women and anemic men, as indicated in **Table 2.3**.

Table 2.3: Calculated iron requirements based on iron bioavailability

World Health Organization versus the Indian Council of Medical Research

	World Health Organization		Indian Council of Medical Research		
	Estimated Basal Requirement (mg/day)	Dietary iron requirements based on low bioavailability diet (5%)	Estimated Basal Requirement (mg/day)	Estimated iron bioavailability based on a mixed cereal diet	Dietary iron requirement based on a mixed cereal diet (mg/day)
Adult male	1.05	27.4	0.84	3%	28.0
Adult female	1.46	58.8	1.50	5%	30.0
Pregnant woman	-	-	3.00	8%	37.5
Lactating woman	1.15	30	1.50	5%	30.0
16-18 yr old boys	1.50	37.6	1.49	3%	49.5
16-18 yr old girls	1.62	62	1.50	5%	29.9
13-15 yr old boys	1.17	29.2	1.24	3%	41.4
13-15 yr old girls	1.68	65.4	1.40	5%	28.0
10-12 yr old boys	1.17	29.2	1.03	3%	34.2
10-12 yr old girls	1.20	28	0.95	5%	18.9
7-9 yr olds	0.71	17.8	0.78	3%	26.0
4-6 yr olds	0.50	12.6	0.55	3%	18.4
1-3 yr olds	0.46	11.6	0.35	3%	11.5
Anemic women	-	-	-	6.7%	-
Anemic men	-	-	-	4%	-

Source: WHO guidelines [1], Indian Council of Medical Research [19]

In a population as wide and diverse as India it is difficult to establish set 'iron bioavailability rates' which accurately reflect true absorption rates for all individuals, but these serve as guidelines for estimation. Some in vitro studies from India indicate that the bioavailability rates vary as much by income than by diet type. One study by Rao et al [65] using in vitro methods on meals reflective of typical diets show that ionizable iron in low vs. high income diets from the same cereal base vary greatly. For example among sorghum diets low income diet patterns resulted in ionizability of 3.3% compared to high income patterns of 6.8%, both higher than the ICMR estimated rate of 2% for sorghum diets but also quite disparate between the two groups. Among rice-based diets socioeconomic differences reflected ionizability rates of 4.3% versus 6.3% (low vs. high income), although a statistically significant difference was not reported for either sorghum or rice.

Use of bioavailability algorithms

There are various methods available to determine the bioavailability of iron in foods. *In vitro* methods generally try to simulate the digestive process and measure ionizable or dialyzable iron using reagents and filters. The use of caco-2 cells is a technique that uses cultured intestinal cells to simulate digestion in vitro and allow the measurement of iron uptake into the cells. In vivo methods include the chemical balance method, rate of repletion studies, and the use of either radioisotopes or stable isotopes. The chemical balance method involves long-term measurements of iron intakes and excretion, and is not suitable for measures of meals or individual foods. Rate of repletion studies involve depleting subjects of iron and measuring repletion, and is only conducted in animal studies. Radioisotopes, like stable isotopes, allow iron to be traced through the body from measured quantities consumed and the concentration later found in red-blood cells, but unlike stable isotopes, radioisotopes present some risk to human subjects. Stable isotopes are safer for human subjects, but measurement tends to be more difficult [59]. Radioisotope studies are considered the gold standard method for determining iron absorption in individuals; however, these studies have many limitations including its high cost and the need for a very controlled laboratory-based environment.

Bioavailability estimates generated from these methods on individual food items or meals are useful but in general, any one food item is rarely consumed alone and meals are rarely ever replicated in their exact proportion. Rather, countless combinations of food items from a wide variety of foods are consumed at any given meal for different people with different food preferences and cultures, and dietary patterns change over time. We cannot estimate the amount of bioavailable iron consumed for an individual without incorporating all the food components in a particular meal. In addition, studies conducted on iron bioavailability estimation reveal that the overall bioavailability of a meal is not just the weighted average of the individual bioavailabilities of the ingredients, since nutrients and anti-nutrients interact with each other in the gut, creating synergistic effects [66, 67]. For example, the iron-enhancing strength of ascorbic acid increases relative to the amount of phytates in a meal, which is in addition to and different than the counter-effect of phytate as an inhibitor [57].

A cost-effective alternative to these lab-based tests is the use of mathematical algorithms that calculate bioavailable iron from iron status, iron intakes and the inhibiting and enhancing factors in the diet. For analysis of diets using large samples, or for diets that are analyzed retrospectively, these algorithms provide the only way to estimate bioavailable iron in the diet. There are various algorithms available for use today which have been in development and refinement since 1978 when Monsen et al [68] published an algorithm using intakes of heme iron, non-heme iron, ascorbic acid, and meat, adjusted for iron status. Since then algorithms have been tested and re-examined on various populations with different diets and have come to include intakes of more factors, including phytates, tannins, calcium, soy and eggs (also inhibitors). In a recent review of the available algorithms tested against a highly-controlled 9-month study using the chemical balance method in a group of religious sisters in the Philippines, all six algorithms [57, 67, 69-72] tested underestimated iron absorption in rice-based diets [73]. However, two algorithms, Monsen & Balintfy (1982) and Hallberg and Hulthen (2001) provided the closest approximations to the actual values and had strong agreement between them. The primary difference between the two algorithms is that the Hallberg & Hulthen algorithm incorporates more factors in the diet and allows for calculating the separate effect of each dietary factor.

Bioavailability algorithms have limitations in their application to dietary datasets. They are subject to the quality of dietary data collected and do require knowledge of iron status, which can be difficult to acquire in large or retrospective samples. Most algorithms require information about single meal intakes, whereas most dietary data are from at best, total recalls within a 24-hour period that are not always broken down into specific meals. It has been generally found that bioavailability algorithms tend to underestimate bioavailability when examined from short time periods (<7 days) [60]. A review by Hunt on the application of mineral bioavailability algorithms cautions using them to estimate absolute absorption levels, but encourages using algorithms to estimate the effects of *changes* in bioavailability [74]. There is a need for further application of these algorithms in order to improve upon current low-cost methodologies for assessing iron absorption in large populations.

Certainly the generalized bioavailability ratios currently offered by the ICMR are not suitable to estimate *changes* in bioavailable iron across time, populations or within diets. This is because dietary patterns have been shifting and it not certain that bioavailabilities within cereal groups have not changed. Current ICMR estimates for bioavailable iron are based on the 5:2:3 ratios set in 1983, 25 years ago [19]. Though they have their limitations, iron bioavailability algorithms offer a feasible method to retrospectively estimate changes in bioavailable iron in the Indian diet.

Conceptual Framework

Of interest in this paper is the impact of recent dietary change in India on intake of dietary bioavailable iron (DBI). It has been shown that diets and income patterns have changed for the average Indian over the past thirty years. The dietary shifts have caused not only changes in consumption of total iron but also in the bioavailability of iron in the whole diet. In general, higher income households are able to diversify diets to attenuate the iron-inhibiting effect of cereal-dominated diets, many of which are based on coarse cereals. Therefore income growth, as well as dietary shifts from production pattern changes, also alters iron bioavailability. However, the overall impact of shifts in iron intake as well as iron bioavailability is not well known. DBI, though not often used as an outcome measure in nutrient analysis, is the best indicator of iron sufficiency in dietary analysis.

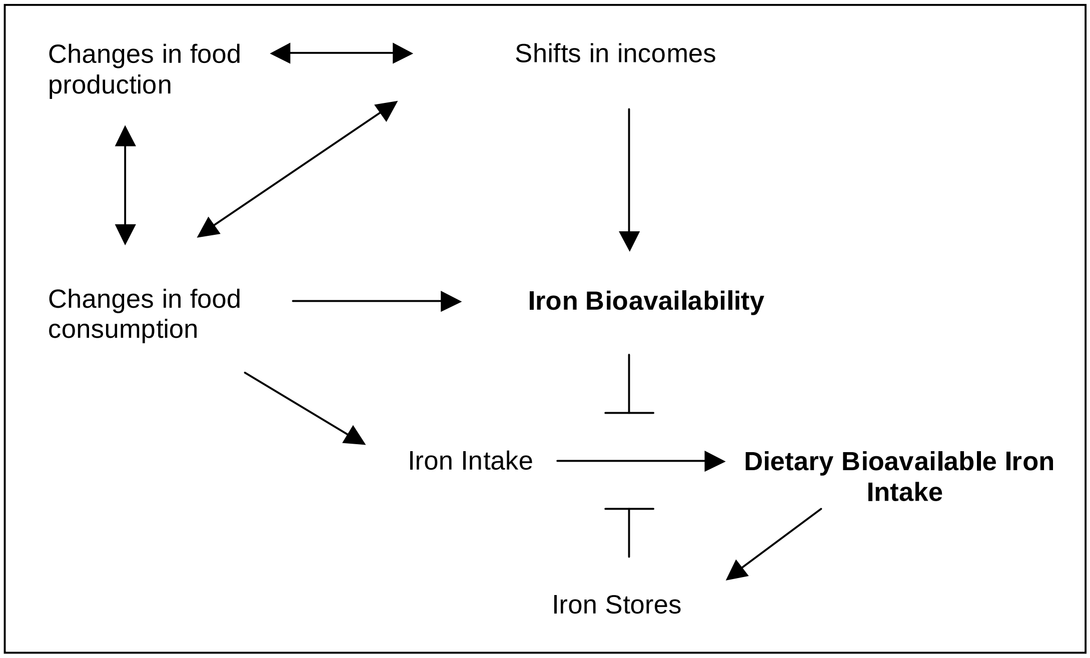

Figure 2.3. Conceptual framework
Shifts in food production increased average incomes, both of which caused changes in food consumption patterns. Changing food patterns altered iron intakes as well as the bioavailability rates for consumed iron. The final dietary bioavailable iron is a product of total iron intakes and the iron bioavailability from the diet. In addition, iron status (iron stores) regulates iron absorption (bioavailability). Trends in iron bioavailability and DBI have not been estimated.

Objective

Given the lack of consistent data on iron deficiency anemia in India, and the persistence of this public health problem, there is a need to examine trends in dietary iron deficiency. Therefore the objective of this research is to determine trends in iron intake, iron bioavailability and bioavailable iron in seven states of rural India over the past 30 years for the general population and within different segments of the population.

Methods

Dataset

Secondary data analysis was conducted on 24-hour recall data from India's National Institute of Nutrition's National Nutrition Monitoring Bureau (NNMB) located in Hyderabad, Andhra Pradesh. The NNMB has collected socioeconomic and dietary data in rural villages since 1975. Four rounds of dietary surveys were available for analysis: 1975-80, 1996-97, 2000-01, and 2004-05. The seven states which had data for all four survey rounds were: Kerala, Tamil Nadu, Karnataka, and Andhra Pradesh in the South, Orissa in the East, and Maharashtra and Gujarat in the West. Dietary data were collected at the household-level as one-day weighted food records, except for every fifth household, where individual-level 24-hour food recalls were collected. Only data from 24-hour recall surveys were used in this analysis in order to best estimate individual-level iron bioavailability. All households received the basic questionnaire and had anthropometric measurements taken on individuals within households. Only rural data were included in analysis. Though urban households were sampled in some rounds, only household-level intakes were collected therefore they were not included. In generally, rural areas of India exhibit anemia rates roughly 10% higher than urban India [37].

The survey design varied over the four survey rounds, spanning 30 years. The sampling frame for the 1975-80 round included 500 households per state selected from four districts (strata), based on development category, and villages within the districts were selected proportional to the population. Households were purposefully selected in this first round to cover all socioeconomic categories. All subsequent samples were taken from the National Sample Survey Organization (NSSO) sampling frame, which divided states into agro-climatic strata. Five villages per strata were selected, with 20 households selected from 5 clusters per village. Roughly 750 households per state were sampled using this new frame. Sampling of caste and tribal samples were reflective of true population ratios. In all rounds, data collection (within each strata, when applicable) was completed in four sub-rounds throughout the year to avoid the effect of seasonality. In order to allow for survey comparability, adjustments for sampling strata and clustering were made for each survey round. State-level population weights were used in all pooled results. Adjustments for cluster sampling were also used on all survey rounds where the cluster variable existed. The cluster variable was unavailable in the 1975-80 round dataset and so a region variable was used as a proxy, resulting in a more conservative estimate of the standard error than either not using a cluster proxy at all or the true cluster variable itself would have been. All results presented below reflect these adjustments for survey design, unless otherwise noted.

Data collected from the National Nutrition Monitoring Bureau is of good quality. There are problems inherent to any dietary data collected including measurement error, recall bias and data accuracy and this dataset is no different. However, few countries have dietary monitoring data that extend back to the early 1970s, as India does, and they have employed rigorous designs and surveys since they began. Data collection enumerators were nutritionists and doctors, both clinical professionals who received additional training in dietary survey methodology. Having teams who understood the importance of accuracy from correct serving portions to triggering information recall and had a local understanding of food cultures were crucial in collecting dietary data as accurately as is possible. The NNMB has systematically collected five-yearly nationally representative data, which are reliable and heavily used and accepted by the nutrition community in India. Their data have been used extensively in research, surveillance and monitoring including various policy and scientific publications internally by the Planning Commission, the Dept of Women and Child Development, state governments and the Nutrition Foundation of India as well as externally by UNICEF, FAO, the WHO and IFPRI (34). One limitation of the dataset is that dietary data were not collected from all states, but rather 7-10 states depending on the willingness of individual state governments to participate. Although the data are used as representative of the country as a whole, the South is heavily represented. The impact of this bias on generalizability to all of India will be discussed later.

Application of the bioavailability algorithm

Dietary bioavailable iron (DBI) was determined by applying the bioavailability algorithm to individual 24-hour recall dietary intake data. Calculations for bioavailability rates of each dietary factor were based on individual level intakes in milligrams for phytate-phosphorus, ascorbic acid, tannic acid and calcium, and in grams for meat/fish/poultry and soy protein. Phytate-phosphorus values were converted from milligrams of phytic acid (where phytate-phosphorus = phytic acid/3.5). The full algorithm is presented in Appendix C based on the equations presented in the Hallberg & Hulthen published algorithm and three subsequent errata [57, 75, 76].

Food composition data Nutrient content values from the Indian Food Composition Table (FCT) [55] were used as the first resource for all values. However when values were missing for specific food items, data from the ASEAN table for use in Asia [77] were used, and if unavailable, then from the USDA food composition online database [63]. Tannin values are not included in the Indian FCT, therefore values were first taken from the appendix in the Hallberg & Hulthen paper [57]. If data were not found in the appendix, data from independent published analyses were used [65, 78]. The breakdown of heme vs. non-heme iron content in meat, fish and poultry items were taken from the Hallberg & Hulthen appendix. The final food composition table used for all analysis can be found in Appendix B. During data cleaning, only extreme outlying values found for recorded consumption of any given food item (defined as >1000g) were removed from the dataset before the algorithm was applied to the data. Finally, food codes were collapsed to allow for comparability across time frames. In 1975, 66 food items were listed in the 24-hour recalls, in 1996, 76 food items were used and finally, after 2001, 625 food items were included. In order to compare dietary changes over time with similar precision the original nutrient composition table of 66 food items was used in nutrient analysis across all 24-hour recalls. Individual food items were collapsed into their original 66 codes and converted using one conversion factor per nutrient and food group, for example all green leafy vegetables entered in 2005 had the same nutrient value for iron.

Serum ferritin adjustment The algorithm incorporates a measure of iron status into the bioavailability equation due to the upregulation of iron absorption in iron deficient individuals. Serum ferritin (SF), a measure of storage iron, is generally used to determine iron deficiency without anemia, although it can be highly elevated in cases of infection, sometimes masking iron deficiency. Serum ferritin <12 μg/L is considered the clinical cut-off for iron deficiency when there is no sign of infection present. However, no measure of iron status was available for individuals in this dataset, and the default estimate in the algorithm (unadjusted SF= 23 μg/L) was thought to be too high for a population with high anemia prevalence, population-wide serum ferritin adjustments were necessary.

A few studies in the mid-1980s in India were conducted to determine mean SF levels and their range among a healthy population from the high socioeconomic strata [79, 80]. Among healthy non-anemic adult women and men, average SF values were 24 μg/L (range: 2.9 μg/L – 70.0 μg/L) and 50.4 μg/L (range: 9.2 μg/L – 115.0 μg/L), respectively, and 30% of the 60 women sampled were iron deficient. Results confirm a consistent difference in iron status between men and women. Among healthy (but not necessarily non-anemic) schoolchildren (5-16 years old) mean SF values were similar in girls and boys (mean and range for girls and boys, respectively: 24.9 μg/L (3.5 μg/L to 97.7 μg/L) and 24.5 μg/L (8.0 μg/L - 133.1 μg/L)). Using NFHS anemia data for ever-married women, children, pregnant women and men (56.2%, 79.2% , 57.9%, and 24.2% as presented in Chapter 2), we estimated that 99.9% of women and children and 61% of men are iron deficient (SF<12 μg/L), based on the assumption that iron deficiency prevalence is roughly 2.5 times the prevalence of anemia in a population [1]. Using these data and the serum ferritin ranges in a healthy population we established mean serum ferritin values for women and children to be 8 μg/L and 11.9 μg/L for men. The algorithm adjustment for a SF value of 11.9 almost doubles bioavailability rates (multiplicative factor of 1.86) while a SF value of 8 nearly triples it (multiplicative factor of 2.7).

Income data

Collection of income data was consistent throughout survey rounds: totaled from all the individuals' reported annual incomes within a household. However, during data entry on the earliest survey round, these data were transformed into categorical groupings as *per capita* daily income. In all later rounds, data were entered and available as continuous variables of *per capita* annual income. Therefore, use of *per capita* income (PCI) measures in analysis was restricted by 1975 data that was collected as a categorical variable. In all other rounds, PCI was entered as a continuous variable. Tertiles were deemed to best fit the data groupings and therefore calculated for the remaining surveys based on 1975 breakdowns: the first tertile included the poorest 37.1% of the population, the second tertile included the middle 33.6%, and third tertile formed the wealthiest 29.2% of the population. There were no hedonic variables in the survey that would allow us to validate or create a proxy for the income variable, and a certain amount of bias is expected in this measure as it is based on recall from one year.

Other variable transformations

Other variable recoding was necessary across the surveys to match response codes. In doing so data were forced to conform to the broadest categories available for each variable. For example where recent surveys offered six categories for religion, the 1975 categories were only four (Hindu, Muslim, Christian and Other), thus Jainism and Sikhism were added under the "Other" category.

Statistical analyses

All statistical analyses and some graphs were completed using Stata 9.2 by StataCorp [81] while other graphs and all tables were made using Microsoft Office Excel. The primary outcome measure in this analysis is dietary bioavailable iron (DBI, in mg/day). Because only secondary data analyses were conducted, sample sizes were not a consideration a priori, however they were in general quite large. The smallest sample was 6,800 individuals, sufficient to detect a .05 mg difference in average dietary bioavailable iron at 90% power (assuming average iron intakes of 14.0 mg in women, a standard deviation of 8.9 mg [11] and 10% iron bioavailability). Secondary outcome measures included iron bioavailability (as a percent of total iron) plus the individual iron absorption ratios for tannins, phytates, ascorbic acid, calcium, meat, eggs, alcohol and soy.

All adult individuals were included in the analysis (n=45,062). Children were excluded in order to get a sense of dietary trends without having to adjust for the percentage of children in each of the subgroups analyzed and how this changed over the period of the analysis. In reviewing 24-hour recall data and in later analyses we see that dietary patterns for children do not differ much when compared to adults. In addition, in younger children dietary intake patterns can be difficult to reconstruct and can lead to high variability in iron intakes. For the purposes of this trend analysis, adult dietary intakes presented the most interpretable summarization of overall dietary trends in the population.

Time trends were plotted to show shifts in bioavailable iron across four time periods 1975-80, 1996-97, 2000-01 and 2004-05, stratified by state, sex and socioeconomic status. As with most dietary intake data, frequency distributions for outcomes measures tended to be skewed. Given the large sample size and the assumptions of the central limit theorem, significant differences in trend analysis were conducted using parametric tests and significance testing was determined using t-tests at alpha=0.05.

Results

A breakdown of sample characteristics can be seen in **Table 2.4**. Vegetarianism is quite high in the population and remained roughly 80% through all survey rounds. Survey rounds mostly differed in their total sample, ranging from 6,918 in 1975-80 to 15,458 in 2004-05. Among states, major differences included a predominance of non-vegetarian diets in Kerala (67%), and a larger than average tribal population in Gujarat (23%) and Orissa (19%).

Trends in cereal consumption within these seven states are shown in **Figure 2.4**. As expected rice is the major cereal in these diets and rice consumption increased dramatically from 1975 to 1996, while sorghum and finger millet consumption declined. Although there was an upward trend in wheat consumption, overall it was quite low, indicative of the bias in the sample towards southern India. Wheat consumption is greatest in the northern states of Punjab, Uttar Pradesh and Haryana, none of which are included in these surveys.

Dietary iron vs. dietary bioavailable iron

Trends in iron intakes show little change from 1975 to 2000 (see **Figure 2.5**), however

Table 2.4. Descriptive Statistics on sampled population

Across seven state over four rounds of National Nutrition Monitoring Board surveys

Frequencies with percent breakdowns per survey round and per state

		By Survey Round[1]						Scheduled			
		1975-80	1996-97	2000-01	2004-05	Female	Non-Vegetarian[2]	Tribe	Caste	Hindu	Total
By State	Kerala	13%	20%	15%	14%	54%	67%	2%	11%	62%	100% **6,833**
	Tamil Nadu	14%	8%	13%	13%	52%	15%	2%	25%	90%	100% **5,509**
	Karnataka	22%	14%	15%	13%	51%	6%	8%	19%	93%	100% **6,928**
	Andhra Pradesh	15%	16%	14%	14%	51%	15%	3%	27%	96%	100% **6,540**
	Maharashtra	15%	12%	15%	15%	51%	9%	10%	21%	87%	100% **6,537**
	Gujarat	14%	12%	13%	16%	50%	3%	23%	14%	95%	100% **6,295**
	Orissa	8%	18%	15%	15%	51%	15%	19%	22%	97%	100% **6,384**
	Female	51%	51%	51%	52%						**44,026**
	Non-vegetarian	20%	16%	16%	15%						
	Total	100% **6,918**	100% **8,435**	100% **14,215**	100% **15,458**						

[1]data collected between 1975-80 and 1996-97 unavailable for analysis

[2]non-vegetarian sample determined from consumption of animal-source food in a 24-hour period

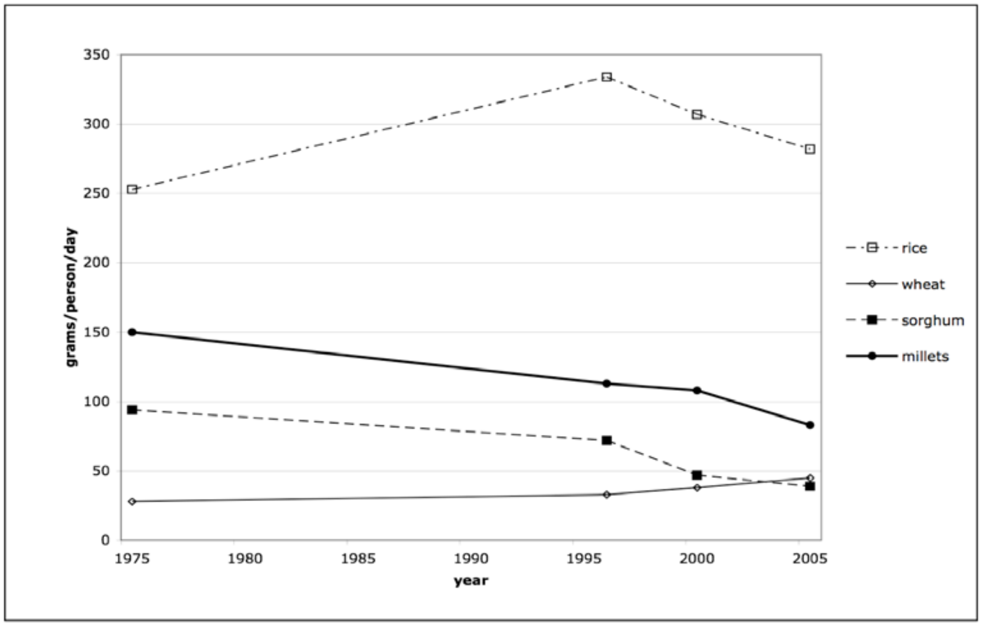

Figure 2.4. Trends in consumption of major cereals (1975-2005)
Among rural adults across seven states of India over four survey rounds, in gm/day
Intake estimated from individual 24-hour recall data (n=45,026)

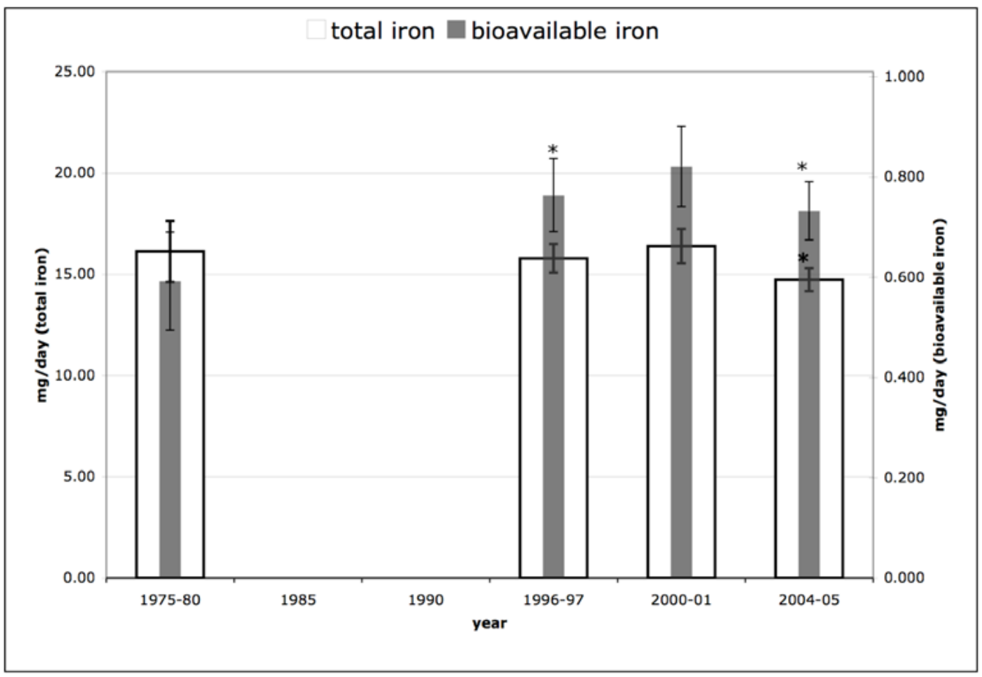

Figure 2.5. Trends in iron intake versus bioavailable iron intake (1975-2005)
Among rural adults across seven states in India, * indicates significant difference from previous year (p<0.05). Axes are scaled to represent similar percentages of average requirements for total and bioavailable iron for this population (e.g. 25 mg/day of iron and 1.01 mg/day of bioavailable iron both represent ~85% of requirements).

intake of dietary bioavailable iron (DBI) increased, indicating improvements in iron bioavailability. Iron intakes remained approximately 16 mg/day per average adult, while DBI increased from 1975-80 to 2000-01 from 0.48 mg/day to 0.67 mg/day ($p=0.005$), on average a gain of 0.01 mg per year. However, in the last five years both total iron intakes and DBI showed significant declines (DBI has dropped to 0.61 mg/day ($p<0.05$)). As reference, the average iron and DBI requirement for this population is 29 g of iron/day (30 g/day for women and 28 g/day for men) and 1.2 mg of absorbed iron/day (from basal recovery mean requirements according to the ICMR for adult males = 0.84 mg/day and for adult females = 1.50 mg/day). Therefore both axes represent similar scales for requirements for the general adult population, where 25 mg/day total iron and 1.0 mg/day bioavailable iron represent 85% of daily requirements. These total iron requirements are based on estimates of 5% bioavailability for women, and 3% for men, therefore an average of 4% for mixed-rice based diets in the population. Estimating sufficiency of intakes from iron intakes would result in different trends than estimates from bioavailable iron sufficiency. Analysis stratified by gender indicated that trends in women's DBI intake do not differ from men's, though they are systematically mildly higher at each time point due to the higher absorption rates women have. Because women's requirements are much higher they still have higher rates of iron deficiency than men.

Closer dietary analysis reveals that these improvements in DBI came from caloric gains over the first twenty years increased (2007 calories/adult/day in1975-80 to 2277 calories/adult/day in 1996-97 ($p<0.05$), while DBI density remained ~0.065 mg DBI/100g diet). (DBI density by weight instead of calories is used here for easy comparison to nutrient content data and because people purchase in grams not calories). By 2000, however, DBI intake improvements came via improved dietary quality (DBI density increased to 0.076 mg/100g diet ($p<0.05$)). And finally, the decline in DBI in the last five years can be attributed to a lower total iron intakes (from 16.4 mg/day in 2000 to 14.8 mg/day ($p=0.001$)), linked to a decline in total calories (2154 calories/adult/day to 1942 calories/adult/day, $p<0.05$), since bioavailability did not increase significantly. The resulting decline in DBI seen from 2000 to 2005 correlate with findings of increased anemia seen in NFHS-III and will be examined further later.

The strongest effect on iron bioavailability in the Indian diet has been from phytates and tannins, which inhibited iron bioavailability by roughly one-seventh (absorption ratio=0.15) and one-third (absorption ratio=0.28), respectively. Calcium has roughly halved iron across all time periods while ascorbic acid doubled and the meat factor, among non-vegetarians, tripled bioavailability. Averaged across the population, the meat factor improved iron bioavailability only by 50%, due to the low prevalence of non-vegetarian diets. The effects of soy, egg and alcohol did not have a significant effect on enhancing or inhibiting dietary iron in these diets at any time point. As trends, the relative effects remain constant over time (i.e. phytates consistently have the strongest effect on dietary iron), but mild changes within absorption factors are seen. Although the overall bioavailability rate remains unchanged, dietary changes from 1975 to 2000 cause a 7% and 6% increase in the enhancing effect of ascorbic acid and meats/fish/poultry, respectively, along with a 10% decrease in the inhibiting effect of tannins. The mild increase in inhibition from higher phytate and calcium intakes wash out the overall effect on iron bioavailability rates in the general population. Further analysis of bioavailability factors is seen disaggregated by income tertile.

DBI by income tertile

An examination of trends by per capita income tertile reveals the true heterogeneity of the population. In 1975 the poorest tertile consumed *more* iron than the richest but by 2005 are consuming significantly less than the richest tertile, due to a steady decline among the poorest tertile since 1975. However, the poorest third also experience simultaneous improvements in iron bioavailability. This inverse relationship between iron intake and iron bioavailability in diets among the poor is apparent in **Figure 2.6**. Iron intakes dropped from 18.4 mg to 13.4 mg from 1975 to 2005 while bioavailability improved from 2.6% to 4.0% ($p<0.05$). Diets of the richest tertile, on the other hand, show improvements in bioavailability without sacrificing iron intakes up until 1995. The unusually high intake of iron among the richest tertile in 2000-01 is due to a surge in pearl millet consumption following a drought. As expected, iron bioavailability of diets among the richest tertile tend to be higher than the poorest tertile, although by 2005 they converge at close ~4.5%.

The net effect of these iron intakes and iron bioavailability can be seen in **Figure 2.7** as DBI intakes by income tertile. Improvements in DBI intake over the first twenty years (1975-1997) were seen in both the poorest and richest tertile, although it was more pronounced among the highest tertile. Any improvement in the middle income tertile DBI intake was delayed until 2000. From 1996-97 onward, diets of the richest tertile show a steady decline in DBI intake back to 1975 levels, from roughly 1.0 mg/day to 0.7 mg/day on average among adults.

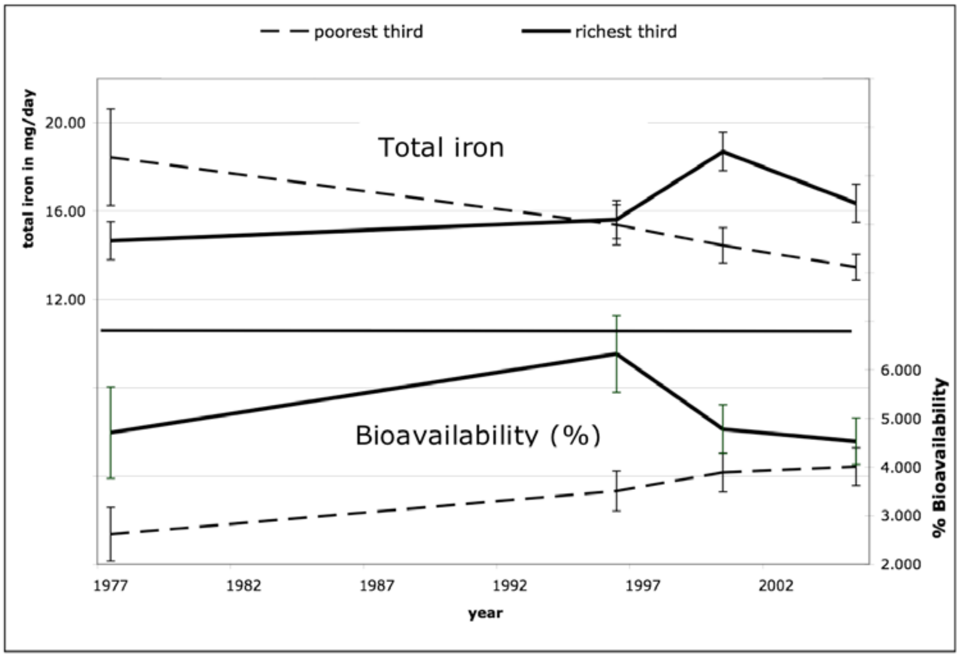

Figure 2.6. Total iron & iron bioavailability by income tertile (1975-2005)
Among rural adults across seven states in India over 4 survey rounds (n=45,026)
Dotted lines represent the poorest tertile of the population, whereas solid lines represent the richest tertile. Error bars are 95% confidence intervals.

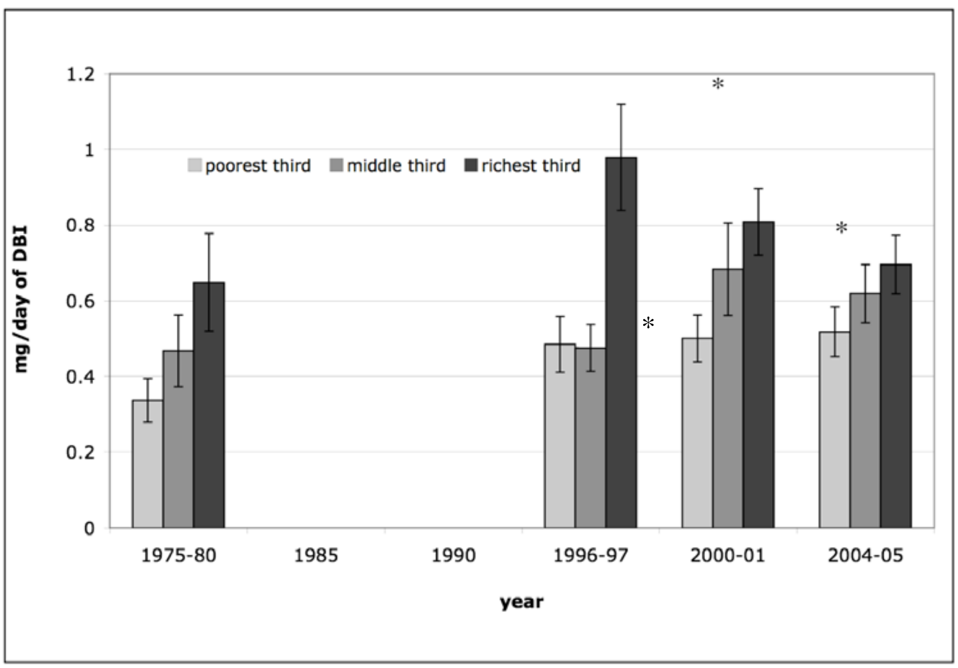

Figure 2.7. Trends in dietary bioavailable iron (DBI) by income tertile (1975-2005) Among rural adults across seven states of India (n=45,026), error bars are 95% confidence intervals and * indicates a significant change from the previous year. The average adult requirement for basal iron is 1.2 mg/day for this population.

Total dietary iron is negatively associated with iron bioavailability (correlation coefficient= -0.492, p=0.008). This correlation signifies that half of the total variation in iron bioavailability can be explained by iron content in the diet, and is likely because the major source of iron in these diets, cereals, are also the main source of iron inhibitors.

An analysis of dietary bioavailability factors, by income, help in understanding why the richest tertile saw uncharacteristic improvements in 1996 and why the poorest diets have steadily improving bioavailabilities. **Figure 2.8** depicts changes in each of the dietary factors by their 1975 levels (as indices). Also though it does not distinguish the final effect of each factor, it does show sources of change in the bioavailability of the diet. Improvements in iron bioavailability among the poorest tertile are from increased ascorbic acid enhancement (from higher intakes) and a decline in tannin inhibition (due to both fewer tannins in the diet, but also from the increase in ascorbic acid which minimizes the effect of tannin inhibition, see algorithm in Appendix C) from 1975 to 1996, and have since held steady. In addition to those improvements, after 1996 mild increases in meat consumption and mild declines in phytate and calcium in the diet contribute to the continuing improvement in bioavailability among the poorest. For the richest tertile, initial improvements in bioavailability seen in 1996 returned to 1975 levels by 2005. The short-term improvement was the result of higher intakes of meats and ascorbic acid and lower intakes of tannins and phytates. In summary, the poorest tertile continue to hold improvements in iron bioavailability seen by the mid-1990s while any dietary quality gains in the richest tertile have been lost, explaining the leveling off in DBI among the poorest and the decline in DBI since 1996 among the richest third of the population.

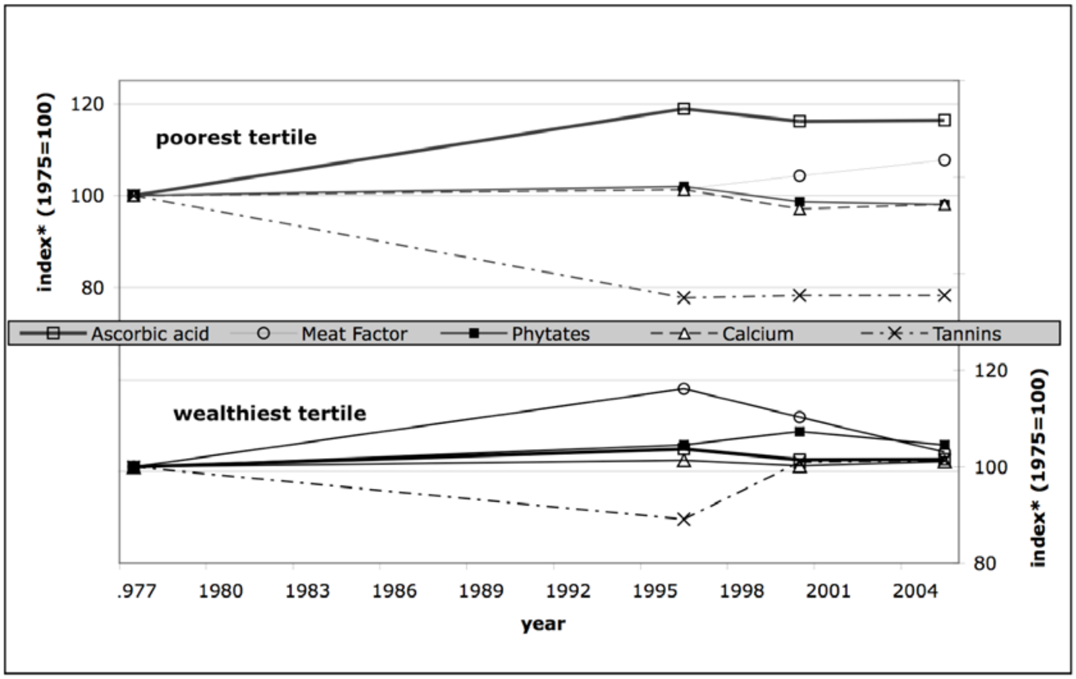

Figure 2.8. Relative changes in iron bioavailability factors by income
Within poorest and richest tertiles from 1975-2005, as change from 1975 levels
*indexed on 1975 levels (=100) for each individual bioavailability factor

The dietary causes of these shifting affects of the bioavailability factors can be seen in **Table 2.5,** which details dietary intakes by per capita income tertile in these four survey rounds. Of concern is the recent drop in DBI among the wealthiest tertile, as well as across the board declines in caloric intakes in the last five years (2000-2005) to all time lowest levels. The sharp increase in DBI among the wealthiest tertile from 1975-80 to 1996-97 is due mostly to higher iron bioavailability from increased consumption of meats/fish/poultry and vegetables (the slight shift from traditional cereals to rice and wheat is very mild but undoubtedly contributed to lower iron inhibition in the diet). In the last five years the richest tertile has increased both dairy and fat intakes, perhaps replacing iron-rich or iron enhancing foods. On the other hand, the poorest tertile has seen steady improvements in DBI from increased consumption of ascorbic acid-rich fruits and vegetables, including green leafy

Table 2.5. Dietary Trends by Highest and Lowest Income Tertile (1975-2005)

Mean intakes among adults[1] with standard deviation[2]

	Wealthiest Tertile				Poorest Tertile			
Mean ***SD***	1975-80	1996-97	2000-01	2004-05	1975-80	1996-97	2000-01	2004-05
Calories(kcal/day)	2005 *40.0*	2220 *24.5*	2270 *23.5*	1934 *19.0*	2050 *49.0*	2267 *24.5*	2021 *17.5*	1909 *18.5*
Iron Factors								
Total iron (mg/day)	14.7 *0.6*	15.6 *0.4*	18.7 *0.7*	16.4 *0.4*	18.4 *1.1*	15.4 *0.5*	14.4 *0.4*	13.4 *0.3*
DBI[3] (mg/day)	0.65 *0.06*	0.98 *0.07*	0.81 *0.04*	0.70 *0.04*	0.34 *0.03*	0.48 *0.04*	0.50 *0.03*	0.52 *0.03*
Iron Bioavailability (%)	4.7 *0.5*	6.3 *0.4*	4.8 *0.3*	4.6 *0.2*	2.6 *0.3*	3.5 *0.2*	3.9 *0.2*	4.0 *0.2*
Food in g/adult/day								
Rice	253 *17.2*	296 *8.8*	289 *9.5*	237 *9.5*	230 *21.5*	358 *12.0*	309 *11.1*	305 *10.3*
Wheat	43 *6.0*	50 *4.6*	54 *5.1*	65 *4.3*	18 *3.5*	27 *4.0*	29 *3.2*	34 *2.3*
Pearl Millet	11 *3.8*	15 *3.6*	63 *8.9*	43 *5.5*	15 *4.0*	13 *3.2*	24 *4.4*	15 *3.2*
Finger Millet	15 *4.0*	12 *2.3*	14 *2.7*	7 *1.9*	72 *18.4*	30 *4.5*	23 *3.4*	25 *3.9*
Sorghum	54 *12.0*	39 *5.5*	30 *4.0*	28 *4.2*	142 *23.8*	84 *9.9*	66 *7.7*	51 *5.2*
Total Cereals	382 *13.0*	431 *6.5*	470 *7.0*	309 *7.5*	493 *14.0*	525 *6.5*	455 *5.0*	365 *9.0*
Pulses	36 *2.2*	35 *1.6*	37 *1.0*	33 *0.9*	28 *2.4*	31 *1.1*	27 *1.2*	28 *1.0*
Green Leafy Vegetables	10 *1.7*	12 *1.2*	11 *1.3*	11 *1.0*	11 *1.9*	19 *1.9*	16 *1.3*	16 *1.3*
Vegetables	55 *4.4*	58 *2.5*	50 *1.7*	56 *2.3*	35 *2.5*	42 *2.7*	41 *2.3*	40 *1.7*
Fruits	24 *3.5*	27 *1.7*	27 *1.4*	31 *1.6*	8 *1.3*	20 *1.7*	16 *1.4*	26 *1.8*
Meats/Fish/Poultry	18 *4.7*	28 *3.3*	22 *2.5*	19 *2.3*	19 *6.5*	9 *1.0*	11 *1.1*	12 *1.5*
Dairy	109 *9.2*	111 *5.9*	111 *3.4*	140 *5.3*	30 *3.0*	48 *3.6*	37 *2.0*	43 *2.3*
Fats & Oils	17 *1.5*	17 *0.6*	15 *0.3*	30 *0.8*	6 *0.5*	12 *0.4*	12 *0.4*	19 *0.7*
Sugars	22 *1.7*	27 *1.6*	19 *0.6*	16 *0.7*	14 *1.8*	18 *0.7*	15 *0.9*	15 *0.8*

[1]adult sample (n= 45,026) across 4 survey rounds

[2]standard deviations for 1975-80 data are generally larger (more conservative estimates) due to inability to adjust for cluster sampling and therefore more robust SE used (see methods section)

[3]DBI=dietary bioavailable iron

vegetables, even despite a significant drop in meat consumption to half former levels.

DBI by state

Large variations are found in state-level results for DBI. It is important to keep in mind that populations found within each of the examined states (30-60 million) are similar to that of many countries and nutrition programs are generally run by the state in India, so state-level analysis is useful. **Table 2.6** details major differences among the seven states examined. Across the seven states there is large variation in dietary patterns, wealth and population. Kerala has the smallest population with only 21 million rural inhabitants compared to Andhra Pradesh, which has almost three times as many rural individuals. Kerala, Tamil Nadu, Andhra Pradesh and Orissa are major rice consuming states, with over 80% of all cereal consumption from rice. The other three states represent diets based in millets, sorghum, wheat and rice. Rural consumers in most states rely heavily on cereals for the bulk of their diet, roughly 60% of total grams of food purchased, whereas Kerala and Gujarat sit apart with only 42-45% of the volume of their diet from cereals. Across all time periods of the survey, Kerala and Gujarat are the wealthiest states, with the majority of their rural inhabitants in the wealthiest tertile of the seven states across all time points. Orissa is consistently the poorest state. Some distinguishing features of the state diets, which also hold across time, are the (1) high consumption of green leafy vegetables (GLVs) in Orissa, where they consume 3-4 times as much as any other state, (2) high consumption of dairy in Gujarat, and (3) high consumption of vegetables in Kerala, Gujarat and Orissa. Although pulse consumption is low across states (~20-30g/day) in Karnataka average intakes were 50 g/day in 1996-97. Notable dietary changes over time within each state are (1) a dramatic decline in millets consumed in Karnataka from 1975 to 2005 from 400g/day to 150 g/day, (2) a tripling in fruit consumption since 1975 in four states, Kerala, Andhra Pradesh, Tamil Nadu and Karnataka (all considered South India) from ~10 g/day to 30 g/day, with Andhra Pradesh in 2005 consuming 45 g/day.

Table 2.6. State characteristics in 2005

Based on dietary recall data, per capita income based on recall, and national census data

State	Rural Population (in millions)[1]	Relative Wealth (rank)	Major Cereal	Reliance on Cereals (% of total diet from cereals[2])	Dietary Characteristics
Kerala	21.4	2	rice	45	high MFP
Tamil Nadu	36.8	3	rice	57	high fruit
Andhra Pradesh	59.1	4	rice	58	high fruit
Orissa	27.4	7	rice	60	high GLV/ high veg[3]/low dairy
Karnataka	31.1	6	rice, millets, sorghum	60	large drop in millets high pulse
Maharashtra	48.4	5	sorghum, rice, wheat, millets	56	mix cereals, no predominant other foods
Gujarat	34.4	1	pearl millet, wheat	42	high dairy/high veg

[1]extrapolated from 2001 census data (www.censusindia.gov accessed 11/15/2007)
[2]by weight
[3]veg denotes vegetarianism

Trends in DBI by states are shown in **Figures 2.9 & 2.10**. As seen in Figure 2.9, Keralans exhibit DBI intakes far above any other state and above the median requirements for the examined population at all time points after 1975. After Kerala, all states at all time points exhibit DBI intakes lower than median requirements (~1.2

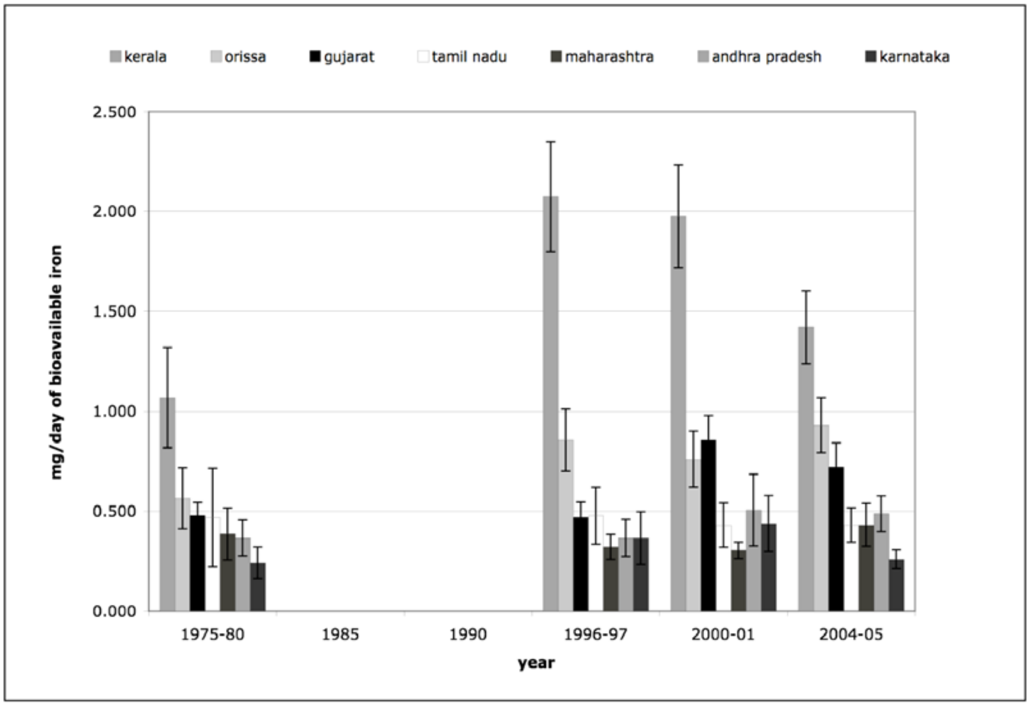

Figure 2.9. Trends in dietary bioavailable iron by state (1975-2005)
Among rural adults in seven states of India (n=45,026)
Three dark colored states indicate non-rice based diets

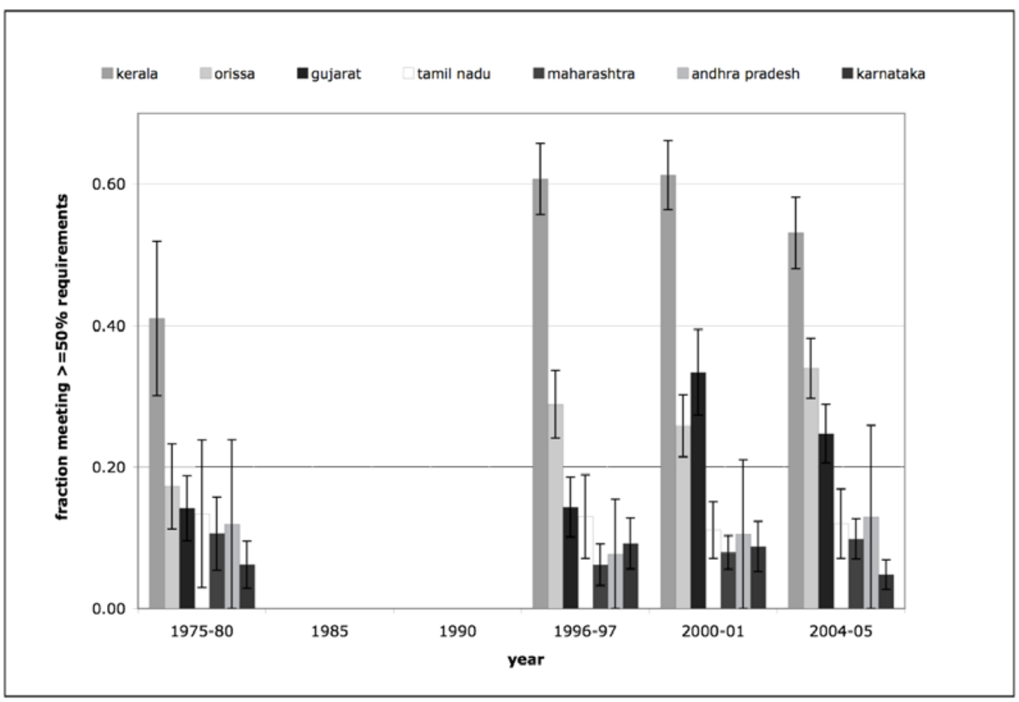

Figure 2.10. Percent of women meeting over half the requirements for basal iron
Among non-pregnant, non-lactating rural women in seven states (n=19,841)
Three dark colored states indicate non-rice based diets

mg/day for this adult population). Orissa and Gujarat exhibit the next highest DBI intakes after Kerala, while Karnataka has the lowest DBI across most time points. Although overall trends show significant improvements in DBI from 1975 to 1996, state-wise analysis shows us that only Kerala and Orissa contribute to this improvement. Then from 1996 and 2000, DBI intakes remain constant except in Gujarat, where it spikes in 2000-01. In the last five years, Kerala and Karnataka have seen statistically significant drops in DBI intake, while Maharashtra shows a mild improvement.

Similar trends are seen in Figure 2.10 when the data are presented as the percent of the non-pregnant, non-lactating (NPNL) women meeting >=50% of their DBI requirements. Kerala has consistently more women meeting their requirements than any other state at any time (between 40%-60% of all women) whereas in all other states less than 40% of women are meeting 50% of the requirements. In Gujarat in 2000-01 again we see a spike, where 32% of women are meeting half their requirements, up from 15% in 1996-97. The variation seen in DBI intakes among NPNL women is pronounced in a state like Andhra Pradesh where the 95% confidence interval shows that between 0% and 22% of women are meeting half their iron requirements.

Comparing these data on NPNL women meeting less than half their iron requirements, (i.e. having low DBI intakes and therefore at risk for iron deficiency anemia) to actual anemia data in these states, both from 2005, we see how our ‘Low DBI’ indicator measures against anemia. Keeping in mind that anemia estimates are not specifically iron deficiency anemia, but rather all cause anemia (other nutrient deficiencies, hemoglobinopathies, etc..), our indicator tests fairly well in Gujarat, Kerala and Orissa, where rank order is maintained, but not as well in the other four states where our estimates show more risk for IDA than anemia prevalence indicates.

See **Table 2.7** for a comparison of these anemia estimates. As a measure of change in risk our estimates are closer and tend to underestimate change from 2000. We show increased prevalence in all states but Orissa, where risk went down 4.5 percentage points. Anemia data show a similar trend in Orissa as well as in Tamil Nadu, which was not picked up by our dietary indicator.

Table 2.7. Comparison of 'Low DBI'[1] indicator with DHS reported anemia prevalence

Absolute values and measures of change since 1999-2000
Among non-pregnant non-lactating women only

	Prevalence estimates 2005		Change since 1999-2000[3]	
State	DBI<50% iron requirements (%)	Anemia Prevalence from DHS data[2] (%)	Anemia prevalence points (%)	Low DBI prevalence points (%)
Kerala	46.9	32.3	9.6	4.9
Orissa	66.0	62.8	-0.2	-4.5
Gujarat	75.3	55.5	9.2	9.5
Andhra Pradesh	87.1	62.0	12.2	1.4
Tamil Nadu	88.0	53.3	-3.2	2.5
Maharashtra	90.2	49.0	0.5	0.4
Karnataka	95.2	50.3	7.9	4.4

[1]Low DBI defined as DBI intakes <50% of basal requirements
[2]Demographic Health Survey Data [13, 14] (1989-99, 2005-06)
[3]negative change indicates lower prevalence in 2005 compared to 1999-2000

The last five years

Further state-wise results can be seen in **Table 2.8,** which presents a breakdown of DBI, iron intakes and iron bioavailability findings in 2005. Kerala exhibits iron bioavailability and DBI levels significantly higher than any other states, across all time points, due to the high consumption of fish in their diets. Results are consistent with DHS findings where anemia prevalence in Kerala was 32% among ever-married women in 2005, the lowest among the examined states. Orissa exhibits the second highest mean bioavailable iron level across all time periods, due mostly to high iron bioavailability rates. Consumption of GLVs in Orissa has a tripling effect of iron absorption from ascorbic acid, as opposed to most states where levels of ascorbic acid at best double iron bioavailability. In general, rice-consuming states (Kerala, Orissa, Andhra Pradesh and Tamil Nadu) have higher rates of iron bioavailability than states consuming mixed cereal (Karnataka) or sorghum/millet based diets (Maharashtra and Gujarat)**,** which is consistent with ICMR's relative bioavailability estimates (the 5% : 2% : 3% ratios for rice: wheat/millet:mixed cereal diets). The high iron/low bioavailability diet in Gujarat provides the highest DBI levels in non-rice consuming diets (0.72 mg/adult/day) and is comparable to Orissa (0.93 mg/adult/day in 2005), where diets exhibit the reverse pattern, a low iron/high bioavailability diet.

Discussion

Overall Conclusions The objective of this paper was to describe the trends in iron intake, iron bioavailability and dietary bioavailable iron (DBI) intake in seven states of rural India over the past 30 years. The persistence of iron-deficiency anemia over the past thirty years despite massive food production improvements warranted a better

Table 2.8. Mean Dietary Bioavailable Iron (mg/day), Total Iron and Iron Bioavailability by State in 2005

Among adults (n=45,026) by sex, income, and change since 2000

	Dietary Bioavailable Iron (*mg/day*) in 2005								
		By Sex[1]		By Income[2]		Compared to 2000			
State	Overall	Men	Women	Tertile I	Tertile III	in mg/day	as %	Total Iron (*mg/day*)	Percent Bio-availability
Kerala	1.42	1.43	1.41	**1.07**	**1.79**	**-0.56**	**-28%**	10.85	11.27%
Orissa	0.93	**0.84**	**1.02**	0.84	1.25	**0.17**	**22%**	11.26	7.27%
Gujarat[3]	0.72	0.73	0.72	0.52	0.66	**-0.14**	**-16%**	26.36	2.64%
Andhra Pradesh	0.49	**0.43**	**0.54**	0.45	0.48	NS	-	10.28	4.12%
Maharashtra[3]	0.43	0.44	0.43	0.38	0.43	NS	-	18.56	2.20%
Tamil Nadu	0.43	0.44	0.43	**0.33**	**0.57**	NS	-	9.94	4.00%
Karnataka[3]	0.26	0.24	0.28	0.25	0.32	**-0.18**	**-41%**	15.27	1.99%

values **highlighted** are significantly different from each other within comparative groups ($p<0.05$)

[1]Differences between men and women include adjustments for different iron status and therefore iron bioavailability rates

[2]Tertile I represents the poorest third or the population, Tertile III represents the richest third of the population

[3]States which do not consume rice as the predominant cereal in the diet (rather sorghum, wheat or millets are the base of the diet)

NS indicates a non-significant value

understanding of not just iron intake but iron bioavailability and DBI intake. This work provides evidence that diet changes in India since the early 1970s have resulted in an overall increase in mean dietary bioavailable iron (DBI) in the diet among rural adults. These improvements came from both caloric increases from food production gains as well as improvements in iron bioavailability, not from an increase in iron intake itself. Recent declines in DBI intakes, however, are cause for concern and may be attributed to consumption of fewer calories seen across the population.

State-level analysis, however, reveals that the overall gains in DBI intake seen from 1975 to 1996-97 were only from two of the seven states. These are Kerala and Orissa, which are both rice-consuming states but represent opposite ends of the spectrum in terms of wealth, infrastructure and rural development. Both states benefited from agricultural growth as rice-producing states that brought income growth and higher food availability. Whereas Keralan wealth and non-vegetarian diets contributed to their high DBI intakes, Orissa's improvements came from caloric gains (and therefore iron intakes) in diets with already relatively high iron bioavailability. Orissa's 'positive deviance', or ability to provide for higher than average DBI intakes despite rice-based poor vegetarian diet seems to be due to their high consumption of green leafy vegetables. Unlike Orissa and Kerala, which exhibit low iron/high bioavailability diets, Gujarati diets consistently provide the third highest DBI intakes from high iron/low bioavailability diets. Among states that have high iron/low bioavailability diet Gujarat stands above the rest because they tend to be wealthier (therefore have slightly higher iron bioavailability rates and caloric intakes), and they consume pearl millet, a cereal that may be protective against low DBI intakes.

The recent decline in mean DBI intake is substantiated by DHS findings of increasing prevalence of anemia from 1999-2005. The rising cost of food grains since 2000 is likely contributing to this decline in DBI. Surprisingly, the decline in DBI witnessed over the past five years is almost entirely amongst the richest tertile. This seems to be due to fats replacing iron-rich or iron-enhancing foods, as we see a doubling in the consumption of fats & oils while overall calories declined.

Using Iron Bioavailability Algorithms This study contributes to the literature testing the use of algorithms in determining iron bioavailability and, although further testing is warranted, we conclude their use holds much promise as the estimates produced are reliable and consistent with *in vivo* estimates. Without the estimation of bioavailable iron, trends in iron intakes alone would have led to very different conclusions on iron deficiency risk in India over this time.

We compare iron bioavailabilities calculated by the algorithm to those established by the ICMR (Indian Council of Medical Research) based on what we know about state consumption patterns. Algorithm-calculated bioavailabilities of wheat/millet diets across all income categories in Karnataka and Gujarat correlate well with ICMR estimates even accounting for income variations, where bioavailabilities range from 1.9% - 2.8% versus the ICMR estimated bioavailability of 2%. Mixed cereal diets across income groups in Karnataka range in bioavailablity from 1.9 %- 3.8% and also compare well with the ICMR estimate of 3% for mixed cereal diets. However, states and income tertiles from rice-based diets vary greatly in iron bioavailability, ranging from 3.5% - 13.3%. ICMR estimates for rice-based diets are 5%, which falls within but fail to reflect the larger variation seen in iron bioavailability in rice-based diets.

Therefore, for non-rice diets the use of the algorithm may not add that much more precision in estimating bioavailable iron, however, for rice-based diets, large variation in diet-attributable bioavailability exists that the ICMR estimates may not sufficiently capture. None of the ICMR estimates allow for an adjustment between non-vegetarian and vegetarian diets. Also, it should be noted that the 5%: 2%: 3% ratio suggested by the ICMR is for adult men (8.3%: 3.3%: 5% for women) and our results are averaged across all adults, therefore they tend to underestimate bioavailabilities. A closer re-examination of our algorithm calculations against diet types may shed more light on the accuracy addition benefit of using the algorithm to predict bioavailability, (as opposed to the ICMR ratios) and will be done in Chapter 3.

The Hallberg & Hulthen algorithm tended to underestimate DBI intake as was found in a previous study [73], but this underestimation was systematic and therefore still valuable in detecting relative changes or differences. Because not all the anemia is due to iron deficiency anemia, our estimates for DBI still seem to be low. There are some explanations for our findings of such low DBIs: (1) our serum ferritin values to adjust the bioavailability which we set on a population-level for women and children separately from men may have been too high, or (3) our nutrient content information for some of our tannin and phytate content may have been higher than actual values. Although the algorithm was tested on low iron vegetarian diets, it was not tested on diets with such high levels of iron inhibitors or among such iron deficient individuals.

The usefulness of the algorithm in iron analysis would depend on the purpose of the assessment and the population under examination. With access to sufficient iron status data on the individuals examined, these algorithms could be more precise.

Further testing and refining of bioavailability algorithms will only add to the existing toolbox of methodologies from which nutritionists can work to improve upon dietary assessments, either on the level of the individual or the population. The lack of expense is particularly an asset in low resource settings. Unfortunately these algorithms also require accurate and high quality data on individual intakes as well as thoroughly tested nutrient and anti-nutrient composition in food, which can be a challenge in resource-poor settings.

Analysis at the level of iron intakes instead of DBI intake (i.e. without the algorithm) would have resulted in very different findings and conclusions. Based on the declining iron intakes among the poor since 1975 we would have concluded that the poorest third of the rural population was at increasing risk of iron deficiency anemia over these thirty years, when in fact they were not. Similarly, we would have concluded that Keralan diets were not providing enough iron. This is true even had we used the ICMR bioavailability rates for rice and calculated DBI, due to the fact that meat/fish consumption is not captured in the 5% estimate. The ability to adjust, at an individual level, for iron bioavailability has allowed more precision in estimating change in DBI intake over time and across groups.

Calculating DBI intake from an algorithm can be a simple program. The most difficult part is collecting high quality dietary intake data and having good food composition data, both of which are required in any nutrition analysis. The lack of data on serum ferritin concentration, or baseline iron status, is not going to change for large-scale analysis and therefore will always be a limitation. Iron status will need to be estimated and therefore removing some of the inter-person variability, but at least the dietary-based bioavailability variation is still maintained this way.

Limitations & Internal Validity Although the data available for analysis were ideal in many respects, they presented some limitations for analyses using the iron bioavailability algorithm. First, mealtimes were not entered in the datasets and therefore all items consumed in a 24-hour period were analyzed as if it were one meal. This likely did not have a significant effect for this study, but if any estimation effect was seen it would be greater for higher income groups where more variety is seen among meals than is seen among the poor who have more monotonous diets [82], or among urban diets and not rural diets. To test the effects of this limitation, on a sub-sample of observations, 24-hour 'meals' were divided into one half and one third to recreate actual meal volumes (many rural consumers only eat two meals a day in India). DBI intake was then calculated and re-summed, then compared to 'one meal' results. Results indicated that there is a marginal dose-related increase in bioavailable iron over a 24-hour period as meal numbers increase. Because the number of meals a day was not available in the dataset, we did not divide total food volumes and therefore may have contributed to the underestimation of bioavailable iron. However, these effects were irrelevant for either trend analysis or the examination of relative differences, as was the objective of the research.

A second limitation to the data available for analysis with an iron bioavailability algorithm was the lack of data on iron status among individuals, since iron stores greatly determine iron uptake. Individual variation in iron status was impossible to capture, beyond our basic estimations presumed solely on gender. Our estimate of 8 mg/dL for women and 11.9 mg/dL for men was tested against estimates at 8 mg/dL, 11.9 mg/dL, and 23 mg/dL for all individuals (men and women). Using all variations of serum ferritin status, absolute values of DBI shifted up or down (higher serum ferritin values yielded lower DBI intake), but trends remained stable. Our imputed values provided the best estimates to actual mean serum ferritin values based on data available for the population. It should be noted that data on both hemoglobin and serum ferritin, even had they been available, still have their limitations in estimating individual iron status. Low hemoglobin, or anemia, as we know, can be due to many factors not just low iron. Serum ferritin, though a good measure in combination with hemoglobin to determine low iron status, is greatly elevated during infection, thereby masking iron deficiency. Given the conditions of rural India, the prevalence of infection-inflated serum ferritin measures would likely have been high had we even had the data available, and therefore would not have been especially useful. A reliable estimate for individual iron status is still needed in the field of iron nutrition.

A third limitation to the dietary data was that the consumption of tea and coffee, both high in tannins, were not recorded as such, but rather as components of milk, water and sugar. Without tea consumption data, the inhibiting effect of tannins is likely underestimated, however because tannins are common in other foods in India and the inhibiting effect shows minimal rate of returns at these high intake levels the impact of additional tea consumption data is negligible. For example, the addition of two cups of tea to each individual's diet only alters the average absorption ratio from 0.28 to 0.27. However, data from research in India on the affect of tea consumption on iron absorption shows that the addition of two cups of tea reduces absorption by between 49%- 67% across iron deficient and iron replete women [83]. Since algorithm-calculated bioavailabilities tended to underestimate true values, we did not adjust for mean tea consumption in any final analyses. However, the additional variation in DBI due to tea consumption differences added to our estimation error and limited our predictability of the error.

Another limitation to the use of the dietary datasets with the bioavailability algorithm was the lack of information about cooking methods and storage time in the dataset. For example, germination and fermentation of foods are common techniques in preparations of some Indian foods (*idly* and *dosa*) and are not captured in the dietary data. Such preparations tend to increase the content of ascorbic acid and degrade some of the phytates, improving the bioavailability of iron in the food. These limitations are cited by the authors of the algorithm and will continue to be a limitation in any application of these algorithms until better dietary data collection methods are available to record food preparation and account for any substantive nutrient gains and losses.

Sensitivity analysis One of the assumptions of the analysis was that food composition table data were accurate. This assumption is particularly important for the iron content of rice as the premise of the work revolves around iron and rice-based diets. Indian FCT values for iron in rice are over twice those found in most other parts of the world, including analyses from Asian rice varieties. To test how sensitive our results were to the assumption of the high iron content of rice, we ran the major outcomes assuming the iron content of rice was only 0.3 mg/100g instead of 0.7 mg/100g. Results from this sensitivity analysis indicate, not surprisingly, that the trends, relative shifts and conclusions remain the same. There is a moderate shift down in all values by an average 0.05 mg/day (or 10%) of DBI intake.

External validity Because the NNMB only collects data in participating states, we had to limit our analysis to the seven states that participated in the 1975 round. Although three other states have since joined the NNMB surveys, they could not be used in analysis as there were no more than two time points available for them. The seven participating states are predominantly in the south and therefore do not accurately represent all of India. Four states analyzed are major rice consuming states (Tamil Nadu, Kerala, Orissa and Andhra Pradesh) while Karnataka, Maharashtra and Gujarat consume a larger variety of cereals. Wheat-based diets, most predominant in the northern areas of India are only represented in part in these analyses. Regardless of country-level representativeness, all the states included in analysis, except Tamil Nadu, have seen an increase in anemia prevalence over the past 5 years, according to the DHS surveys [13, 14], and are therefore relevant for analysis of trends in iron deficiency. However, recent shifts in cereal prices favored rice over wheat, therefore post liberalization dietary changes may have been very different in the north where wheat is more commonly consumed than in the states analyzed. Conclusions from this work do not extend to wheat-consuming states in India.

Implications and explanation of mechanisms Closer examination of the DBI intake spike in 2000-01 seen in Gujarat shows a surge in pearl millet consumption at this time. In January 2001 an earthquake hit the state which affected over 35 million individuals according to WFP reports [84]. The spike could be due to the massive food aid donations to Gujarat in 2001 following this natural disaster, but may also be due in large part to a shift to iron-rich, drought-resistant pearl millet after a series of drought that affected the region from 1999-2001. Data from Gujarat in 2000-01 indicate a 100 g/day increase in pearl millet consumption over 1996-97 and 2005 diets. It is interesting that this crisis-induced pearl millet consumption was likely protecting individuals from developing greater iron deficiency in this time of crisis, in fact doubling the number of NPNL women meeting half their iron requirements. The contribution of pearl millet diets to DBI intakes will be examined in more detail in Chapter 3.

Orissa proves that wealth is not necessary for high DBI intakes, but their high anemia rates indicate that perhaps endemic poverty may be contributing to other causes of anemia, like hookworm or malaria infection. Another possible explanation for Orissas high anemia rates is their large tribal population, among whom genetic thalessemia is more prevalent. Other discrepancies between our 'Low DBI' indicator and anemia data may be caused by our assumptions of iron status to be the same across all women in all states. Karnataka, Maharashtra, Tamil Nadu and Andhra Pradesh all exhibit anemia rates more disparate than our indicator than other states (30-40 percentage points apart). In these states, which also have the lowest DBI intakes, iron status of women may be lower and therefore their serum ferritin imputed values in the algorithm too high, causing lower than expected bioavailability rates.

Policy implications Even considering a modest underestimation of DBI calculated using the algorithm, the values for DBI intake are still far below levels required, and help explain why iron deficiency in India is as endemic as it is. Short-term recommendations to improve bioavailable iron in the Indian diet should be geared towards the promotion of vitamin C-rich foods in the diet during mealtimes, ideally through the consumption of green leafy vegetables. Further reduction of tannins and phytates in the diet may not be the best solution as they also have positive health effects in the diet as antioxidants (tannins) and for their association with dietary fiber (phytates). Long-term recommendations to improve bioavailable iron in the Indian diet should also incorporate efforts to improve overall iron intakes. This might include efforts like iron biofortification of staple crops, which will be discussed in Chapter 4.

Dietary quality improvements in the rural population, rich and poor, may be partially due to the opening of the economy and the economic growth, which followed from agricultural growth. Although improving micronutrient intakes was not an objective of the agricultural intensification of rice production in India during the 1960s, these results provide evidence that in increasing per capita cereal supplies, and thus lowering prices and saving incomes, some diet quality improvements can be achieved- for the rich and the poor, above and beyond caloric gains. This begs the question of whether macro-level agricultural production policies need to consider the direct effects of micronutrient malnutrition, as theorized by the authors. Although this only examines bioavailable iron, would all micronutrient-level nutrition inadvertently be positively impacted by agricultural growth? Are such considerations only important in times of shortages? The drought and earthquake crisis in Gujarat resulted in *improved* DBI intakes from pearl millet consumption. The current crisis of rising food grain prices, will likely continue the downward trend we see in calories and thus DBI, having negative implications for combating anemia. It is concerning to note that average caloric intakes in 2005 are lower than they were even in 1975 in the midst of a

Malthusian crisis. The recent decline in caloric intake is of concern beyond the level of iron deficiency, and warrants political action.

It could be argued that the methodological shift in the collection of dietary recall from 66 food groups in 1975-80 to a disaggregated 625 food groups by 2000-01, as explained in the methods section, could explain these caloric differences. However, the greatest drop in caloric intakes occurred from 2000-01 to 2004-05 when 24-hour recall methodologies were identical.

Future Research Current estimates for bioavailable iron are still based on the 5:2:3 ratios set in 1983, 25 years ago [19], despite changing consumption patterns and the widening disparity found between income groups and from rural to urban diets. Given the changes in dietary composition in the Indian diet over the last 40 years a re-estimation or re-categorization of iron bioavailabilities in the Indian diet should be considered. Evidence that shows bioavailability rates differ greatly by income further supports the idea that more individualized determination of bioavailablity may be useful in dietary assessment. Some re-classification of ICMR bioavailability estimates should be considered.

This work applies a bioavailability algorithm to dietary data to better understand dietary shifts and their implications for iron deficiency. Although results tended to underestimate DBI intake, the usefulness of the algorithm cannot be overstated. A further refinement of bioavailability algorithms for the developing country context would prove invaluable for researchers and dietary monitoring staff in countries, like India, where combating iron deficiency is essential to progress.

India has amongst the highest rates of iron deficiency anemia in the world, and nearly every woman and child in the country can be considered iron deficient. According to WHO, the high prevalence of anemia in India is classified as a problem of "severe public health significance." Few gains have been made in preventing or reducing iron deficiency in India, perhaps because it has one of the highest dependencies in the world on plant foods for the diet [85]. This examination of dietary change - at the level of bioavailable iron - helps shed light on the iron deficiency problem in India and why it persists today.

CHAPTER 3

IRON BIOAVAILABILITY AMONG CEREAL-BASED DIETS

Introduction

Iron deficiency in India continues to be a large problem in a country with anemia prevalence rates among the highest in the world. Based on anemia rates, estimates for iron deficiency prevalence indicate nearly every women and child has some level of iron deficiency. In a predominantly vegetarian population, the major sources of iron in the diet are from cereals. Unfortunately cereals also provide the majority of iron bioavailability inhibitors in the diet. The choice in cereal-base of a diet determines, to a large part, the amount of dietary bioavailable iron an individual will consume.

Background

Food production in India

In the 1940s and 50s South Asia was facing the threat of widespread famine from its unprecedented population growth while still dependent on imports for basic staple crops. "Green revolution technologies" introduced modern methods of agriculture mostly through high-yielding variety (HYV) seeds for rice, wheat and maize, which were aided by government policies including price supports for rice and wheat, subsidized water for rice farming, and increased availability (often through subsidies) of fertilizers. These technologies and policies were put forth in an effort to meet increasing demands for food and encouraged many farmers to prioritize rice or wheat production, depending on their agronomic environment, over other crops. The use of HYV seeds, combined with increased land area, tripled the production of wheat and rice from 1960 to 1990 [43, 45]. The emphasis on these cereals crops not only allowed India to avert famine and attain caloric self-sufficiency for a growing population but also resulted in enough surpluses for the country to start exporting cereals by the 1990s.

Few dispute the importance of the green revolution in averting famine, but there are critics who cite deleterious side effects of this agricultural boom in India including excessive use of inputs (herbicides, insecticides, and fertilizers) contributing to environmental pollutants, unsustainable production levels, reduced genetic diversity and depleted soil nutrients through overproduction and mono-cropping [45, 86-88]. Others contend that food insecurity and malnutrition levels still remain high among the poor despite small gains seen in reducing underweight prevalence in preschool age children [45, 86].

Although the agriculture sector only represents one quarter of the country's gross domestic product, it generates income for roughly 60% of the population [42]. Among the rural population, income benefits from agricultural growth were concentrated in areas where irrigation, suitable agricultural climates and green revolution technologies allowed for greater productivity [43-45].

Cereal production patterns in India

India represents the second most populated nation in the world with roughly 1.1 billion people, all of whom live in a land area just over one-third of that of the United States. The most fertile agricultural zone in the country is part of the Indo-Gangetic Plain, which extends from Pakistan through India and covers 7 Indian states that stretch across the north-central region from Punjab to West Bengal. The large Ganges River, fed by the Himalayas flows through this area and impacts positively on soil fertility and irrigation water. This region is also called the 'breadbasket of India' and, in terms of productivity gains, benefited most from the green revolution. Rice is planted in the rainy season (kharif) and wheat in the drier winter season (rabi) and irrigation is used when necessary. High wheat consumption is common in the Western region and rice in the Eastern areas, although both are now grown throughout the Gangetic Plain. The East is a very rural region apart from the city of Calcutta (Kolkata), and is composed of 5 states including West Bengal and Orissa. This area is predominantly small-farmer holding and reliant on tropical rains for rice cultivation. The Western region is very dry, mostly desert areas bordering Pakistan and includes the states of Rajasthan, Gujarat and Maharashtra. Drought resistant crops are grown here including sorghums and millets, as well as some wheat and many of the pulses for consumption across the country. This is considered a mixed cropping system zone, in areas where irrigation is available the major cropping system is rice-wheat. Finally, the South has 4 states at the tip of the Indian subcontinent: Tamil Nadu, Kerala, Andhra Pradesh and Karnataka. The southern states of India are predominantly rice consuming, depending on two rainy seasons and/or irrigation for rice-rice (double rice) cropping. Kerala stands apart from the rest of India as being more developed than most other states in India. Their health indicators (including infant mortality, maternal mortality, female education) are far better than the national average and often as good as developed countries [39]. India is as diverse as its terrain, representing a broad spectrum of agricultural zones, diets, religions, and languages. In rural diets, consumption is tied heavily to local production options, and although rice can be bought across India in markets, markets are not always accessible and/or prices limit consumption among some families. The Public Distribution System (PDS) does distribute subsidized rice and wheat (as well as sugar and oil) to qualifying households across the country. It is hard to generalize about a country that has a population larger than the continent of Africa, where each individual state of India is comparable to an individual country not only in population size, but also in the variation of culture, food and language. An individual's cereal-base, especially in rural areas, links them to their land, production and economy.

Dietary change in India

Although wide variations exist in dietary patterns across India, 60-70% of daily energy supplies come from cereals, regardless of socioeconomic status [40]. One of the noted side effects of the green revolution was that traditional cereal crops like sorghum, millets, and pulse crops declined in per capita consumption due to the almost doubling of the population coupled with the lack of disease-resistant, high-yielding varieties and the availability of irrigated land necessary to bolster its production [48, 89]. From 1965 to 1987 per capita consumption of pulses halved, from 61 g/day to just 33 g/day [90, 91], yet the recommended pulse intake from the National Institute of Nutrition is 40 g/day, based on protein requirements in a vegetarian diet [19]. In response to such shifts in food production, some argue that in growing with only calories in mind, nutrient quality has suffered, possibly creating a resurgence of micronutrient deficiencies among the poor [88, 89, 92]. Their arguments are supported by an ACC/SCN report in 1992 that the percent of iron in the food supply was decreasing in South Asia through the 1970s and 80s [3]. However, total iron is not always correlated with bioavailable iron and more needs to be understood about particular diets within India and how bioavailability of iron can be maximized.

Iron nutrition

As seen above, iron intakes declined by about 20% from 1975 to 1996. Most dietary iron in India comes from cereals due to its heavy consumption in a predominantly vegetarian population [55]. Data from ICRISAT Village Level Survey on dietary intakes from rural Maharashtra and Andhra Pradesh indicate that 73% - 82% of iron comes from cereals[56]. The cereals most commonly consumed in India are rice, wheat, some maize and the coarse or traditional cereals of sorghum and millets. Coarse cereals like sorghum and millet are roughly four times higher in iron than rice, gram for gram. Despite the iron content of traditional cereals, according to the National Nutrition Monitoring Bureau (NNMB) in rural areas in 1997 only 5.6% of household surveyed were consuming at or above the recommended intake for iron (28 mg/CU/day) [49]. But iron intakes alone are not sufficient to determine risk of iron deficiency, for iron bioavailability can vary greatly. This is illustrated by the fact that Kerala, with a population average consumption of 12.8 mg/CU/day of iron has a much lower prevalence of anemia than Gujarat, a state where on average 22.5 mg/CU/day are consumed, although part of this could be due to non-nutritional factors contributing to anemia, like parasitic infections.

Iron bioavailability in the Indian diet

The ICMR estimates average iron bioavailability in the Indian context for three different types of diets: rice, wheat/millet or mixed cereal-based diets. Their relative bioavailabilities are estimated at 5%, 2%, and 3%, respectively [19] based on the content of iron-inhibitors in each of the cereals. A breakdown of the content of iron versus iron inhibitors can be seen in **Table 3.1** below. Because rice has fewer tannins and phytates, its iron and the iron provided in the rest of the diet is more available for absorption than iron from diets based on the other cereals.

Table 3.1. Comparison of iron and iron inhibitor content in major Indian cereals

	Iron (mg/100g)	Phytate (mg/100g)	Tannin Equivalents (mg/100g)[1]	Calcium (mg/100g)
Rice (milled)	0.7	288	0	10
Wheat (whole, flour)	4.9	795	23	45
Pearl Millet	8.0	494	13	42
Finger Millet	3.9	732	360	344
Sorghum	4.1	602	77	25
Bengal gram	6.3	497	38	71
Pigeon Pea	2.7	595	50	73

source: NIN Nutritive Value of Indian Foods unless otherwise indicated
[1]tannin content data is from appendix in Hallberg & Hulthen 2001 [57]

In developing iron bioavailability estimates, the ICMR adjusts iron absorption based on an individual's physiological status (as a proxy for iron stores) since low iron status causes upregulation of iron absorption. For example, it is estimated that a pregnant woman (whose iron status is expected to be low) will absorb 13.3%, 8% and 5.3% of the iron in rice, wheat/millet and mixed cereal diets, respectively. These bioavailabilities were determined using extrinsically labeled iron and calculated from mean iron absorption in typical Indian meals (many of which are vegetarian). Estimates were developed for various sub-populations including children of different ages and gender, lactating women, pregnant women and anemic men (see Chapter 2, Table 2.3).

Although millets tend to have lower iron bioavailability this does not always mean that the bioavailable iron they provide is less. Agte et al [66] found that dialyzable iron from whole diets based on pearl millet were 3-4 times higher than the same diets based on rice. Even though pearl millet alone had a lower dialyzable rates than rice (3.1% vs. 5.6%) the total iron in pearl millet compensated for the lower rates, both when consumed alone or in combination with a surrounding diet. The variability in bioavailability rates is not presented in the ICMR estimates, and therefore it is unknown by how much the surrounding diet can increase or decrease total bioavailable iron within these diets.

Iron bioavailability algorithms provide nutrition researchers with a method to determine how much iron that is consumed is bioavailable to an individual, given the whole diet not just the cereal-base. Though they have their limitations, iron bioavailability algorithms offer a feasible method to estimate how dietary changes affect bioavailable iron in the Indian diet.

Objective

The primary objective of this paper is to determine how cereal-based diets compare in providing for dietary bioavailable iron (DBI) given their varying iron and iron inhibitor content, as well as within the context of their surrounding diets. The secondary objective is to simulate dietary modifications within cereal-based diets, which would improve overall DBI intakes.

Methods

The primary outcome measures include estimates of dietary bioavailable iron (DBI) intakes from each cereal-based diets, as well as their risk of creating an iron deficient individual. Given the predominance of rice in the Indian diet due to the influence of the green revolution, we were interested in seeing how rice-based diets compared to other cereal-based diets in providing for sufficient DBI, and therefore contributing to the risk of iron deficiency.

Data included four cross-sectional survey rounds of dietary recall data from rural adults living in seven states of India. Specifically, 24-hour recall data were analyzed from rounds in 1975-80, 1996-97, 2000-01 and 2005-05 (n=45,026). DBI intakes were calculated using the Hallberg & Hulthen iron bioavailability algorithm using intakes of iron as well as iron enhancers and inhibitors in the diet. Inhibitors included in the calculation were phytic acid, tannic acid, calcium, soy and egg. Enhancers included in the algorithm were ascorbic acid, meat/fish/poultry and alcohol. See Appendix C for the complete algorithm. Nutrient and anti-nutrient content data used for the calculations were taken from the Indian Food Composition table (see Appendix B). Data for tannin content were not available and therefore collected from various secondary sources. Iron content of rice was taken from the Indian Food Composition table (0.7 mg/100g rice) although most rice content data from other countries indicate average content closer to 0.3 mg/100g rice. For a full description of the application of this algorithm to dietary data please refer to the methods section in Chapter 2.

To standardize DBI intakes, we defined an individual at risk if DBI was less than 50% of an individual's estimated basal requirements by sex, age and physiological status as defined by the Indian Council of Medical Research [19].   In as much as the authors could determine, dietary bioavailable iron has never been used as a screening tool for iron deficiency, therefore this particular cut-off has not been tested for its ability to correctly classify iron deficient individuals. Dietary screening tools for iron deficiency are generally tested again anemia (given that it is an easier test and a more severe deficiency), come from a US domestic perspective and use less quantified measures like frequency of consumption of indicator foods (like juice) [93, 94]. Although basal losses are already a mean estimate and therefore closest to an EAR (estimated average requirement) which is preferred for use in population studies, we felt that day to day variation in iron intakes was large enough that overall risk should only be considered when a day's diet provided less than half the average bioavailable iron required.

Diets were separated by cereal base as rice, wheat, sorghum, finger millet, pearl millet, maize, rice-mix or mixed cereal diets, if 80% or more of the total cereal consumed came from one cereal (by weight). A 'mixed rice' diet was defined as 50% -80% of the total cereal consumed coming from rice and a mixed cereal diet was defined as having no predominant cereal. Reference diets for logistic regression were rice-based diets. In order to better understand the effect of these cereal diets relative to another in providing for DBI, we regress cereal-based diets on our cut-off for low DBI (DBI<50% of basal requirements for iron).

The theoretical framework for the effect of cereal-base on intakes of dietary bioavailable iron is described here. As cereals provide the bulk of iron and iron inhibitors in Indian diets, we want to know how cereals compare. Confounding effects on this relationship include meat consumption, income and time. The strong effect of meat consumption can greatly improve DBI through providing both heme-iron but also the enhancing effect of MFPs and must be included in analysis so we know we are just estimating the effect of the cereal-base regardless of vegetarianism which seems to be less associated with rice diets. Incomes must also be controlled for as they allow for dietary diversity, which may enhance absorption of iron above and beyond the role of any individual cereal. Finally, all survey rounds are included in this analysis to determine if the impact of each cereal on DBI is consistent across time, as this is a biological determinate we would expect this to be the case, but trends indicate a shift in DBI (see Chapter 2) over time as well as shifts in the diet base. Other variables to be tested in the modeling include state and community. Accessibility to markets, prices of cereals and cultural trends would be picked up in state dummies if these impact the relationship of cereal diet on DBI.   Community is the variable that identified households as being of a scheduled tribe or caste. These socially ostracized groups that do not have access to the same rights and opportunities as ‘forward castes’ in India. Also, all interaction possibilities were tested to see if there were any modifying effects among these relationships.

In order to determine dietary recommendations within cereal-based diets, a subsample of nonpregnant women from the poorest tertile in 2005 (n=2823) were included in a separate analysis because they constitute a high-risk population to whom dietary recommendations should be focused. Only dietary intakes for 2005 were included so that dietary recommendations could be made based on the most recent dietary patterns. In analyses, 442 of the subsampled women consumed any meat/fish/poultry (MFP), which is 16% of this population. Even small quantities of meat (from averaged diets) would have a large impact on DBI, therefore they were separated in analysis. Finally, curves were constructed for each diet and food item to understand the impact of increased consumption on provision of DBI. Change in DBI was calculated given increased intakes in food groups (green leafy vegetables, pulses, meats, dairy, other vegetables, fruits, nuts and tubers), gram for gram above the average intake for that population. These increases were distributed across foods within each food group, in the proportions they are consumed. For example, in calculating a fruit intake increase of 10g for a population consuming half of their fruits by weight in lime juice, then 5 g of lime juice would be calculated in addition to the 5 g of fruit distributed across the remaining fruit groups. The purpose of these graphs would be to provide dietary recommendations by each diet type to see (1) how foods contribute to or detract from bioavailable iron and (2) the relative effectiveness of different foods on improving bioavailable iron intakes. Bioavailable iron intakes are standardized based on estimated average requirements (EAR), which is 1.5 mg/day for women based on average losses. Non-linear results were expected for most food groups due to the interactive effects seen among iron inhibitor and enhancers. These methods were chosen because dietary recommendations are most helpful when the context of the surrounding diet and consumption patterns are taken into account.

Statistical Analyses All statistical analyses and some graphs were completed using Stata 9.2 by StataCorp [81] while other graphs and all tables were made using Microsoft Office Excel. T-tests were used to determine significant differences among diet types in mean DBI.

For the logistic regression, odds ratios for risk of low DBI (defined as consuming DBI <50% of basal iron requirements according to age, sex and physiological status) were estimated for each cereal-based diet compared to rice-based diets (wheat, pearl millet, sorghum, finger millet, rice mix, mixed cereals, maize and 'other' cereals). Control variables included state, community, time (year), per capita income, and vegetarianism. All variables were tested for collinearity *a priori,* and all combinations of interactions were tested in modeling. Due to the nature of complex survey design adjustments, log likelihood ratios cannot be calculated, and therefore the adjusted Wald statistic was used to test model fit. Collinearity was tested using the VIF (variance inflation factor) test where any variable contributing >5 to the VIF would be considered problematic.

In the sub-analysis among NP women, standard regression analyses to model the effect of dietary change on DBI were not used for a few reasons. First, in individual-level analysis many of the analyzable food groups had zero value intakes and therefore would bias the regression coefficients for those continuous variables. Collapsing these foods into larger categories resulted in significant loss of information and we felt would compromise the usefulness of the analysis. Second, theoretical knowledge of potential interactions between food groups on bioavailable iron made for over 100 interaction terms, greatly complicating interpretation of results. Therefore, in addition to the problems inherent in dietary analyses, like heavily skewed iron intake distributions, and those mentioned above, we chose to simulate the effects of dietary change on DBI on ‘average diets.’ Average food intake for individual food items (66 total) was only included if the average value was significantly different than zero ($p<0.05$), and therefore total caloric intake from the ‘average diet’ of each cereal base was underestimated. In this analysis food groups included: cereals, pulses, green leafy vegetables (GLVs), other vegetables, fruits, nuts, dairy and meats/fish/poultry (MFPs).

Results

For analysis on the impact of cereal-based diets on dietary bioavailable iron (DBI) a sample of all rural adults for whom dietary data were collected was used (n=45,026). A breakdown of sample characteristics is presented in **Table 3.2**. Most notable shifts in characteristics from 1975-2005 include a decline in the incidence of non-vegetarian diets. It should also be noted that sample sizes increased significantly over the survey rounds and representation by state differed somewhat. All results are adjusted for state population weights as well as cluster and stratified sampling.

Cereal consumption choices identify consumers as much as religion in India. Diets associated with each cereal can be very different, as they often reflect agricultural zones with distinct crops adaptable to those agro-climates. Agricultural technologies of the green revolution improved production of rice enough to supply more and more affordable rice in non-rice growing regions, increasing consumption. In **Table 3.3** the

Table 3.2. Descriptive statistics of the sampled population

Across seven state over four rounds of National Nutrition Monitoring Board surveys
Frequencies with percent breakdowns per survey round

		Survey Round (Years)				
		1975-80[1]	1996-97	2000-01	2004-05	
State	Kerala	868 *13%*	1656 *20%*	2099 *15%*	2210 *14%*	
	Tamil Nadu	969 *14%*	640 *8%*	1913 *13%*	1987 *13%*	
	Karnataka	1524 *22%*	1188 *14%*	2166 *15%*	2050 *13%*	
	Andhra Pradesh	1010 *15%*	1356 *16%*	2008 *14%*	2166 *14%*	
	Maharashtra	1022 *15%*	1043 *12%*	2094 *15%*	2378 *15%*	
	Gujarat	988 *14%*	1032 *12%*	1855 *13%*	2420 *16%*	
	Orissa	537 *8%*	1520 *18%*	2080 *15%*	2247 *15%*	
	Female	3565 *51%*	4401 *51%*	7395 *51%*	8086 *52%*	
	Non-vegetarian[2]	1318 *20%*	1764 *16%*	2260 *16%*	2682 *15%*	
	Total	**6,918**	**8,435**	**14,215**	**15,458**	**45,026**

[1]data collected between 1975-80 and 1996-97 unavailable for analysis
[2]non-vegetarian sample determined from consumption of animal-source food in a 24-hour period

survey design-adjusted percent of individuals falling within each cereal-based diet is presented from 1975 through 2005.

The data reflect the shift toward rice consumption from 1975 to 1995 in both rice-based and rice-mixed diets, up 14 percentage points from 52.6% to 66.7% total in these two groups, with the rice-diet predominating. These shifts seem to have come

Table 3.3. Trends in cereal-base among consumers in seven rural states of India
Percent of individuals in each diet type by survey round (1975-2005)

Cereal-based Diet[1]	1975-80	1996-97	2000-01	2004-05
Rice	42.0%	57.9%	54.4%	52.9%
Wheat	4.0%	1.7%	3.2%	5.0%
Pearl millet	2.4%	2.2%	5.6%	5.0%
Finger millet	4.5%	0.9%	0.4%	0.4%
Sorghum	13.8%	5.5%	4.5%	4.0%
Rice-mix	10.6%	13.8%	12.3%	12.7%
Mixed cereal	22.7%	18.0%	19.6%	20.0%

[1]Diet considered based on one cereal if 80% or more of the cereal consumed comes from one cereal (by weight). Mixed rice indicates that 50-80% of total cereal consumed comes from rice (by weight), and mixed cereals is defined as having no predominant cereal and <50% of total cereal coming from rice.

from consumers of wheat, finger millet and sorghum diets. The percent of the population consuming pearl-millet based diets did not change significantly over this time.

Distinctions among diet types can be seen in **Table 3.4,** where prevalence of non-meat diets and the percent of the population in the poorest tertile are shown. All non-rice diets show prevalence of vegetarian diets all at or above 95%. The rice and rice-mixed diets are more associated with meat consumption. This is not entirely an income issue since consumers of wheat and pearl millet-based diets tend to be on average wealthier, and have more than a third of their population in the richest tertile. Kerala, because of its relatively small population, represents only 7% of the rice-based

Table 3.4. Some characteristics of diets by cereal-base

Among rural adult consumers in across seven states in India

Cereal-base[1]	Percent consuming Vegetarian Diets	Percent in Poorest Tertile[2]	Percent of adults meeting 50% of basal iron requirements[3]
Rice	75.9	33.6	26.3
Wheat	95.7	25.3	26.0
Pearl Millet	96.3	22.8	41.8
Finger Millet	95.9	61.1	4.9
Sorghum	96.3	52.5	9.8
Rice Mix	83.2	31.9	18.3
Mixed cereals	94.6	32.2	16.1

[1]Diet considered based on one cereal if 80% or more of the cereal consumed comes from one cereal (by weight). Mixed rice indicates that 50-80% of total cereal consumed comes from rice (by weight), and mixed cereals is defined as having no predominant cereal and <50% of total cereal coming from rice.
[2]tertile indicates the poorest third of the population
[2]basal requirements estimated by age, sex and physiological status for average daily iron loss

diets. Consumers of finger millet tend to be the poorest, similarly more than half of the sorghum consumers are in the poorest third of the overall population.

From the food composition table data presented earlier (Table 3.1) we know that cereals with higher iron content also tend to have more iron-inhibiting compounds. We present the mean DBI for each cereal source below in **Figure 3.1**, for adult diets in 2005. Pearl millet diets provide more DBI than any other individual cereal. All cereal types provide less than the basal requirements for adults (1.2 mg/day). Finger millet and sorghum diets, the former not very common, provide the least DBI. Sorghum is most common in Maharashtra, where 22% of the population consumes sorghum-based diets. Consumers within these two diets types also tend to be the poorest, as we saw in Table 6. Rice and rice mixed diets provide between 0.6 and 0.8 mg/day for the average adult consumer, although we are not sure how much is due to the effect of

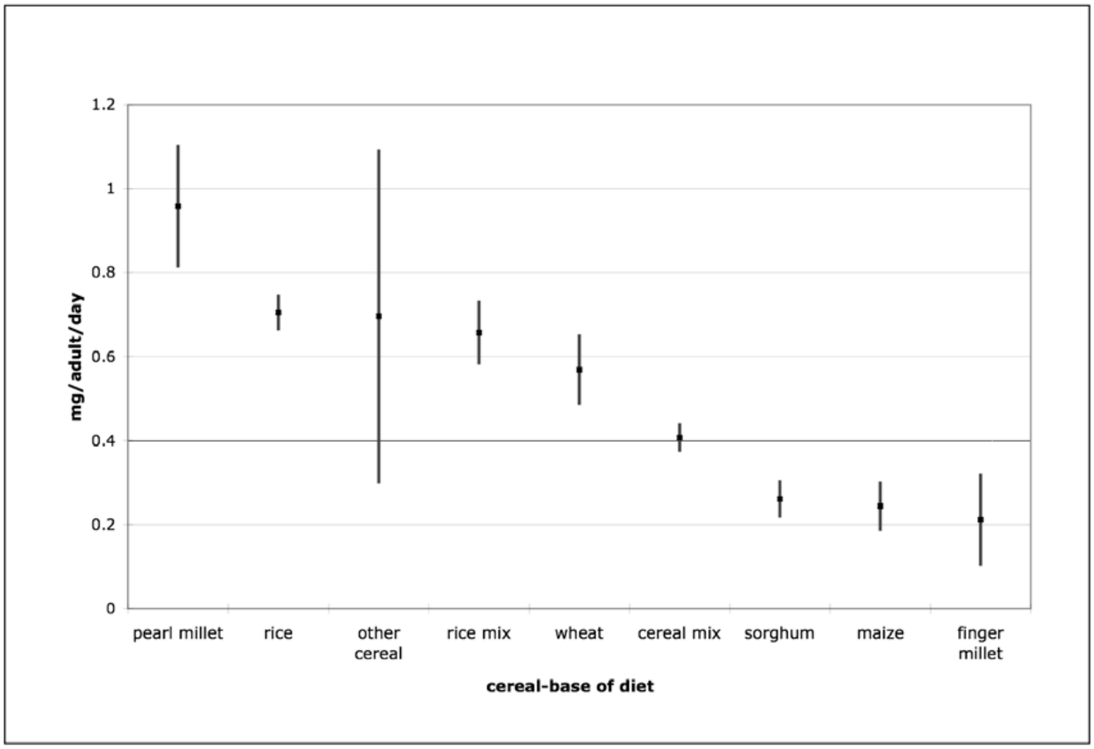

Figure 3.1. Mean intake of dietary bioavailable iron by cereal-based diet
Among rural adults in seven states in India in 2005 (n=15,458)
Bar indicates 95% confidence intervals, cereal base defined by >80% of total cereal by weight from any individual cereal, rice mix = 50-80% from rice, cereal mix has no predominant cereal, 'other cereals' include minor cereals not widely consumed

meat consumption, which is not representative of the larger rice consuming population that is vegetarian.

Regression analysis Community (caste and tribe) did not contribute to the explanation of the relationship between cereal-base and DBI and was therefore not included in the model. Interaction tests were not significant even for income and meat consumption, determining that both were independent effects that did not vary from one cereal-base to another. There was no collinearity problem among the co-variates based on an examination of variance inflation factors for each variable.

The best model fit was found when time, income, meat consumption, and state as well as cereal-base were regressed on DBI. The community variable, although not collinear to income, does not contribute significantly to the model independently or interacted with cereal-base. Because we suspect community acts through income, and it did not add to the model, we dropped this variable from the model. Including state in the model switches the effect of both finger millet and sorghum to protective (when compared to rice based diets) and all states show significant odds ratios when included in the model. There was no collinearity found between state and cereal-base. Therefore state was included as an independent effect in the model, as a dummy variable to help explain differences in DBI.

Results for the regression analysis can be seen in **Table 3.5**. Wheat, pearl millet, rice-mix, mixed cereal and 'other cereal' diets are less likely to result in low DBI than rice-based diets, regardless of income, state, year or consumption of meat. Sorghum-based and finger millet-based diets were not significantly different than rice-based diets in putting their consumers at risk for low DBI. Consumers of pearl millet-based, wheat-based and other cereal-based diets are respectively, 86%, 67%, and 78% less likely to create risk of low DBI than rice-based diets. Other cereals included in analysis include minor millets, as well as barley, but consumption is limited in the general population.

The risk of low DBI was roughly 40% lower by 1996-97 than in 1975-80 and did not change significantly thereafter. When compared to Kerala, the state with the lowest prevalence of anemia, all states except Orissa increased the risk of consuming low

Table 3.5. Odds Ratios for Risk of Low DBI[1] by Diet

Among rural adults based on 24-hour recall data and from algorithm-calculated dietary iron bioavailability

Covariates	Odds Ratios	95% CI
CEREAL-BASED DIET[2]		
(reference = Rice diet)		
Wheat	**0.33**	(0.24 - 0.45)
Pearl Millet	**0.14**	(0.10 - 0.19)
Finger Millet	0.82	(0.43 - 1.56)
Sorghum	0.73	(0.52 - 1.01)
Maize	**3.85**	(2.23 - 6.63)
Other cereal	**0.22**	(0.08 - 0.60)
Mixed Rice	**0.68**	(0.58 - 0.80)
Mixed Cereals	**0.55**	(0.45 - 0.68)
INCOME		
(reference = Highest tertile)		
Middle Tertile	**1.19**	(1.05 - 1.34)
Lowest Tertile	**1.42**	(1.25 - 1.63)
NON-VEGETARIAN DIET[3]	**0.03**	(0.022 - 0.030)
YEAR		
(reference=1975-80)		
1996-97	**0.63**	(0.49 - 0.81)
2000-01	**0.59**	(0.46 - 0.75)
2004-05	**0.60**	(0.47 - 0.76)
STATE		
(reference=Kerala)		
Tamil Nadu	**2.16**	(1.67 – 2.79)
Karnataka	**3.71**	(2.87 – 4.79)
Andhra Pradesh	**2.60**	(2.07 – 3.27)
Maharashtra	**3.08**	(2.27 – 4.16)
Gujarat	0.77	(0.58 – 1.01)
Orissa	**0.41**	(0.32 – 0.53)

[1]'Low DBI' defined as Dietary Bioavailable Iron (in mg) <50% of basal requirements

[2]Diet considered based on one cereal if 80% or more of the cereal consumed comes from one cereal (by weight). Mixed rice indicates that 50-80% of total cereal consumed comes from rice (by weight), and mixed cereals is defined as having no predominant cereal and <50% of total cereal coming from rice.

[3]indicates any meat/fish/poultry recorded in 24-hour recall

[4]due to complex survey design adjustments log likelihood ratio unavailable, therefore we used the adjusted Wald statistic Z=154.07 (p<0.0001), n=45,026

DBI. Orissans were 60% less likely to have low DBI diets than Keralans. These results are surprising given the high poverty levels in Orissa, however bioavailability rates in Orissa tend to be higher than average (7.3%) due mostly to high consumption of green leafy vegetables. Results for income indicate there is increased risk of low DBI as income drops one tertile. Non-vegetarian diets are 97% less likely to provide low DBI than vegetarian diets and is the single greatest protective factor for reducing risk of low DBI. Pearl millet consumption is the second most protective factor.

We tested our indicator of 'Low DBI' from one day 24-hour recall data for prediction of true anemia cases, using a subsample of non-pregnant, non-lactating women in 2005 who had hemoglobin concentration information (n=4625). We defined anemia as Hb<13 g/dL in men and Hb<12 g/dL in women, and our indicator 'low DBI' as DBI intakes <50% of mean basal requirements. Sensitivity of the 'Low DBI' test from one day meal recalls for anemia was 56.9%, specificity=54.0%. This means that 57% of true anemic individuals had 'low DBI' intakes, whereas 54% of non-anemic individuals indeed did not have 'low DBI' (i.e. they had DBI intake >50% of their basal requirement). The ability of 'low DBI' to correctly identify an anemic individual is fairly good (PPV=63.2%), whereas when DBI is not classified as 'low' based on our cut-off, only 27% of the individuals are truly not anemic. The test for the classifications was significant using the survey-adjusted Pearson's chi-squared test (p<0.0001). We tested a higher cut-off (<100% of basal requirements) and found sensitivity to shift to 89.4% and specificity of 11.7%, although the indicator was too high to result in significant results in the classification estimates (Pearson chi-squared statistic adjusted for survey design F=1.06 (p=0.304)).

The bioavailability rates calculated from the Hallberg algorithm are within range of those suggested by the ICMR. In **Table 3.6** we also compare our algorithm-calculated bioavailabilities to bioavailabilities from studies done both *in vitro* and *in vivo* on Indian diets.

Table 3.6. Comparing Estimates of Iron Bioavailability from Cereal-based diets[1]
Algorithm-calculated iron bioavailabilities versus *in vitro* and *in vivo* estimates

Diet Base	*In vivo* as Radio-labeled iron			*In vitro* as Dialyzable iron	**Algorithm-Calculated Bioavailability**	
	Rao 2007 [95][2]	ICMR[3] estimates from typical meals		Agte et al 2005 [66]		
		Men	Women		**Men**	**Women**
Rice	3.6%	5%	8.3%	7.3%	**5.1%**	**6.6%**
Wheat	2.2%	2%	3.3%	-	**2.1%**	**2.8%**
Pearl millet	-	2%	3.3%	9.2%	**1.9%**	**2.5%**
Finger millet	1.6%	2%	3.3%	-	**0.5%**	**0.9%**
Sorghum	1.7%	2%	3.3%	-	**0.9%**	**1.3%**
Rice mix	-	3%	5%	-	**3.3%**	**4.4%**
Mixed	-	2%	3.3%	-	**1.5%**	**2.1%**

[1]All analyses conducted from whole meals using various methods. Algorithm-calculated bioavailabilities breaks down cereal base as >80% of the cereal consumed coming from one cereal (by weight). Mixed rice indicates that 50-80% of total cereal consumed comes from rice, and mixed cereals is defined as having no predominant cereal and <50% of total cereal coming from rice. Other comparative bioavailabilities came from small controlled lab studies and only included the cereal under observation with surrounding diets (averaged over various diets).
[2]analyses were conducted on men and women and averaged
[3]Indian Council of Medical Research [19] from radio-labeled iron studies

We find that our algorithm-calculated estimates compare well with the ICMR recommended estimates and *in vivo* results. The algorithm underestimates bioavailabilities compared to ICMR estimates, but there is consistency across diet types in relative absorption rates. Therefore although the absolute values of DBI we calculate should be used with caution, they are reasonable estimates for absorbed iron. The poorest concordance is found from our iron bioavailability estimates for finger millet diets, which we find to be only 0.6% while in vivo studies are 1.6%. Reasons for our potential underestimation may come from cooking methods associated with finger millet which were not captured on the 24-hour recall data, maybe reducing phytate or tannin content through fermentation and other forms of phytate degradation. *In vitro* estimates, which tend to be higher than *in vivo* estimates, show a higher dialyzable rate for pearl millet diets, which is inconsistent with not only our data but also ICMR and available *in vivo* data. Although pearl millet ranks well in DBI it is due to its high iron content not high bioavailability rate.

Our sub-analysis on women in the poorest tertile is aimed to provide dietary recommendations to the most vulnerable adult population. Only data from 2005 were included to reflect current dietary patterns. Non-vegetarians and Keralans were excluded given their low risk for iron deficiency, and the strong modifying effect of meat/fish/poultry. For example, even in the poorest tertile average intakes of DBI are 2.14 mg/day for non-vegetarians, roughly 43% above basal requirements. Exclusion of Kerala was based on the fact that poorest tertile Keralans on average consume 50 grams of fish and that they skew findings for the average rice-diet consumer. Average diets for each subgroup of women analyzed are presented in **Table 3.7**. Pearl millet diets provided the highest amount of DBI (0.44 mg) followed by rice diets (0.33 mg).

Finger millet diets provide the most calcium, energy and vitamin B12 but the least amount of DBI. Again, pearl millet and rice-based diets provide the most DBI, but

Table 3.7. Average diets & nutrient intakes among poor[1] vegetarian[2] women in 2005

Diet Type[3]	Rice	Wheat	Pearl Millet	Finger Millet	Sorghum	Rice-mixed	Mixed
Sample size	*1160*	*71*	*60*	*16*	*144*	*322*	*450*
Food intake (g/day):							
Rice	427	15	10	15	9	273	112
Wheat	2	297	2	0	2	31	73
Pearl millet	0	0	284	0	0	4	28
Finger millet	3.6	2	0	502	0	66	58
Sorghum	0.5	1	0	0	318	48	99
Pulses	26	26	25	34	27	37	32
Green Leafy Vegs	23	5	10	30	9	10	9
Other vegs	46	31	20	10	28	30	22
Fruits	31	15	2	20	12	27	21
Dairy	38	49	36	90	34	48	54
Nutrient intake (as % of Recommended Daily Allowance as defined by the ICMR[4]):							
Energy	83%	68%	64%	92%	67%	83%	77%
Calcium	67%	79%	69%	507%	59%	122%	120%
Beta-carotene	12%	7%	8%	10%	7%	7%	6%
Vitamin C	123%	50%	46%	60%	46%	70%	55%
Folate	105%	155%	166%	140%	112%	114%	130%
Vitamin B12	8%	7%	5%	16%	6%	7%	10%
DBI (as percent of EAR)	25%	17%	29%	9%	12%	14%	15%
DBI (mg/day)	0.38	0.25	0.44	0.13	0.19	0.21	0.22

[1]poor defined as in the lower third of households by per capita income
[2]not consuming any meat/fish/poultry in 24-hour recall survey
[3]Diet considered based on one cereal if 80% or more of the cereal consumed comes from one cereal (by weight). Mixed rice indicates that 50-80% of total cereal consumed comes from rice (by weight), and mixed cereals is defined as having no predominant cereal and <50% of total cereal coming from rice.
[4]Indian Council of Medical Research from Nutritive Value of Indian Foods [55]

still insufficient amounts. It is not surprising that B12 levels are so low considering this is a vegetarian subpopulation. The Indian Council of Medical Research claims that vitamin B12 deficiency is not a large problem in India, stating that contaminant B12 (i.e. B12 found in bacteria in food products) provides sufficient intakes [19], however there is likely to be some significant deficiencies in the population.

Vitamin A (beta-carotene) intakes are also very low where ~10% of RDA is met by any diet. It is interesting to note that folate, another nutrient implicated in causing anemia and commonly found in whole grains, GLVs and pulses, seems to be consumed in adequate quantities across these cereal-based diets, even among the poorest tertile. Non-vegetarian diets report folate intakes of 83% of requirements, less than any vegetarian diet. Although iron deficiencies is still the major cause of nutritional anemia in India, B12 and vitamin A are also likely substantial contributors.

The effect of food items on improving DBI in average diets among vegetarian, non-pregnant women can be seen in **Figure 3.2**. The strong enhancing effect of adding flesh foods to the diet across all vegetarian diets is not surprising. Roughly 50 grams of MFP (meats/fish/poultry) added to the average diet of poor women would provide

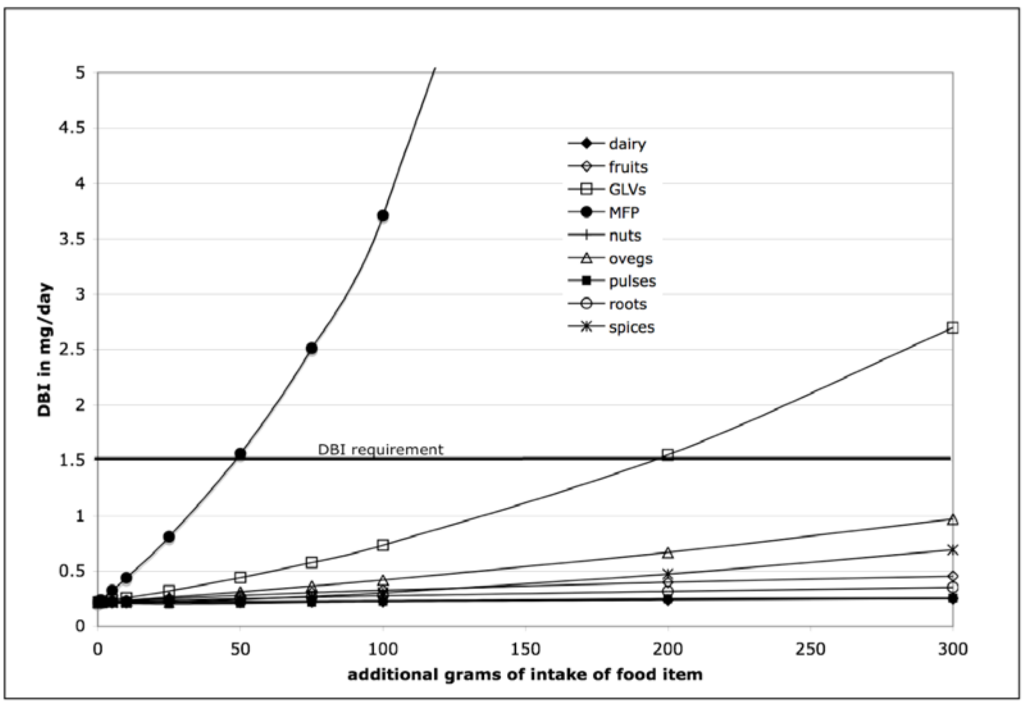

Figure 3.2. Relative effects of foods on increasing DBI among vegetarian diets
Among women in poorest tertile across seven states of India in 2005 (n=2823)
DBI= Dietary bioavailable iron

sufficient DBI, a 6-fold increase from mean DBI intake. Other foods are much slower to improve DBI intakes, but they are nonetheless of significance. For both economic and social reasons, most women in the poorest tertile in rural India only have vegetarian options for improving DBI. After MFP, green leafy vegetables (GLVs), other vegetables, condiments/spices and fruits had the greatest impact on improving DBI. Only GLVs could substantially improve DBI intakes to recommended levels within a reasonable volume of additional food (<300g). The effects of each food group can, however, vary greatly depending on the cereal-base of the diet. This is because even though the food group adds the same amount of iron, it can impact the overall bioavailability of the iron from the cereals as well. For example, in **Figure 3.3** we see the impact of GLVs on DBI within each cereal-based diet.

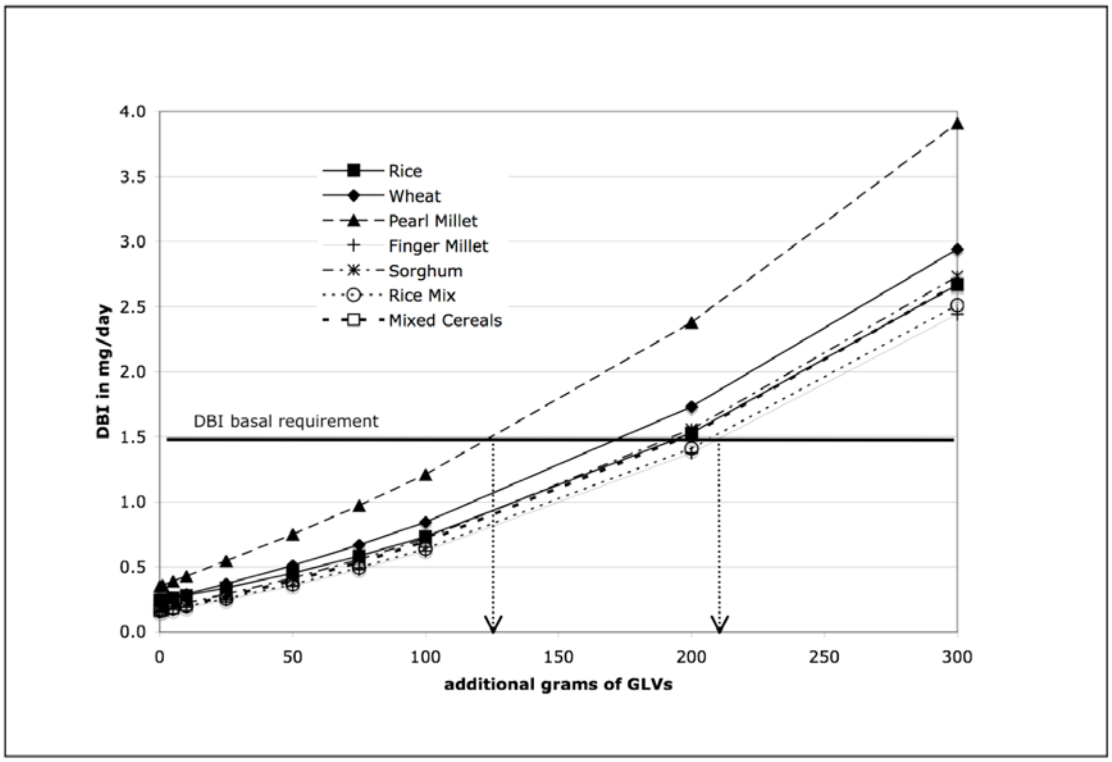

Figure 3.3 Effect of incremental GLV consumption on DBI by cereal-based diet
Among non-pregnant vegetarian women in poorest tertile in 2005
DBI= Dietary bioavailable iron

The addition of green leafy vegetables to the diet improves DBI fastest in pearl millet and wheat diets because the total iron in these cereals is so high. The improvement in bioavailability is similar across cereals, however the rates of improvement are neither parallel nor linear. In pearl millet diets, 150 grams of GLVs added to the diet will provide average requirements for most women. Current poor pearl millet consumers consume only 10 g a day. The improved effect of GLVs on pearl millet diets is due both to the higher starting DBI (0.435 mg vs. 0.203 mg) but also to a steeper slope or rate of improvement gram for gram- due to the underlying total iron in the diet in addition to the improvement in bioavailability.

When dietary simulations are run on all different food groups, an estimation of the quantity of food added to the diet to meet requirements of vegetarian non-pregnant women can be calculated. Results are presented in **Table 3.8 & 3.9**, as the amount of any food item necessary to reach 50% & 100% of basal iron requirements, respectively. Additional increments of 1 g, 5 g, 10 g, 25 g, 50 g, 100 g, 200 g & 300 g were simulated. Any increment over 300g was considered not a plausible increase in the diet for any given food group. Increasing GLV intakes across all diet types by an additional 200 grams per day would meet mean iron requirements in all diets. Only GLVs, other vegetables, and fruits were able to improve DBI to either 50% or 100% of DBI in any cereal-based diet. Given the caloric deficiencies of 2005, the addition of more cereals (of each type) was also simulated, but none of the cereals improved necessary absorption levels to at least 50% within 300g. GLVs, fruits and vegetables tend to be the least expensive foods in India, especially GLVs which are often produced in home gardens and are not often produced as cash crops. Unfortunately GLVs are also often considered an inferior food, some varieties consumed only in

Table 3.8. Necessary additional intake to provide 50% of DBI requirements among poor[1] vegetarian[2] women in 2005
Value is listed as missing if >300g of food needed to meet requirement

Diet Type[3]	**Rice**	**Wheat**	**Pearl Millet**	**Finger Millet**	**Sorghum**	**Rice-mixed**	**Mixed**
Sample size	*1160*	*71*	*60*	*16*	*144*	*322*	*450*
Grams of additional food above average diet							
Pulses	-	-	-	-	-	-	-
Green Leafy Vegetables	200	100	50	200	200	200	100
Other vegetables	300	200	100	300	300	300	300
Fruits	-	-	100	-	-	-	
Dairy	-	-	-	-	-	-	-

[1]poor defined as bottom tertile (third) of the population
[2]vegetarian defined as having consumed no meat/fish/poultry in last 24 hours

Table 3.9. Necessary increase in intake to provide 100% of DBI requirements among poor[1] vegetarian[2] women in 2005
Value is listed as missing if >300g of food needed to meet requirement

Diet Type[3]	**Rice**	**Wheat**	**Pearl Millet**	**Finger Millet**	**Sorghum**	**Rice-mixed**	**Mixed**
Sample size	*1160*	*71*	*60*	*16*	*144*	*322*	*450*
Food group (g/day):							
Pulses	-	-	-	-	-	-	-
Green Leafy Vegetables	200	200	200	300	200	200	200
Other vegetables	-	-	300	-	-	-	-
Fruits	-	-	300	-	-	-	-
Dairy	-	-	-	-	-	-	-

[1]poor defined as bottom tertile (third) of the population
[2]vegetarian defined as having consumed no meat/fish/poultry in last 24 hours

times of hunger or economic hardship.

Results in **Figure 3.4** indicate that among vegetarian diets of women, an increase in current assortment of pulse foods has an initial non-positive effect on DBI except in rice-based diets (and non-vegetarian diets, results not shown). This affect does not seem to have anything to do with the types of pulses being consumed in the rice diets over the other diets as all diets consume predominantly pigeon peas (i.e. red gram) with an assortment of others. This addresses an earlier concern on the affect of the decline in pulse consumption over the period of the green revolution. The additional iron pulses provide cannot overcome for the inhibitors they contribute to the diet, in fact reducing DBI initially. However, given sufficient intakes of pulses the effect can become positive, although alone and in reasonable amounts cannot provide vegetarian women in the first tertile of income with sufficient DBI. Analysis on the subsample of women for whom hemoglobin data is available indicate that mean pulse consumption does not differ between anemic and non-anemic poorest tertile women (28.8 g/day vs. 26.7 g/day, $p>0.05$). The non-vegetarian population consumes half the amount of pulses as vegetarians, so it is possible that pulses may be a substitute for meat, as a protein source, but clearly cannot compete in providing DBI.

Discussion

The primary purpose of this analysis was to determine how different cereal-based diets

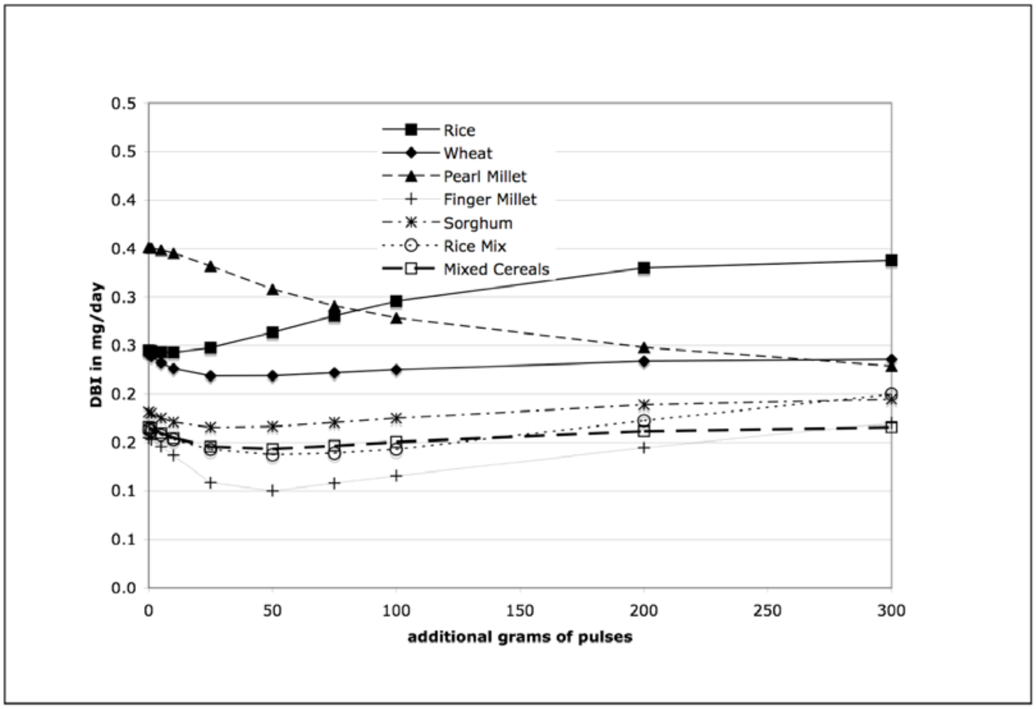

Figure 3.4. Effect of pulse consumption on DBI by cereal-based diet
Among non-pregnant vegetarian women in poorest tertile in 2005, additional consumption of pulses above average intake
DBI= Dietary bioavailable iron

in India provide for DBI intake. Our results on the regression of cereal diets on risk of Low DBI allow us to see that in fact all cereals (except maize), are protective of 'Low DBI' when compared to rice once we adjust for income, vegetarianism and state. Pearl millet and wheat-based diets are shown to provide the most DBI, whether due to the cereals themselves or the diet associated with them. The magnitude of the differences in odds ratios were larger than expected especially after controlling for income tertile and meat consumption. Meat consumption is the single strongest predictor of consuming sufficient DBI, which is not surprising. For the most part, vegetarianism cannot be altered (although many people don't consume meat in India simply because it is unaffordable), but general improvements in wealth and dietary diversity in rural India would have the greatest impact on improving iron bioavailability than certainly shifting to a rice-based diet. The middle tertile and poorest tertile are 20% and 40%, respectively, more likely to consuming low DBI intakes than the richest tertile, regardless of meat consumption or cereal-base. Of the diets, pearl millet diets provide the greatest amount of DBI regardless of income, and a consumer of pearl millet regardless of meat consumption or income is 90% less likely to consume low DBI intakes than rice consumers. The strong results indicating that rice-diets place consumers at more risk for Low DBI compared to the other major cereals would not have been the conclusion had we not controlled for income. It seems that it is not the rice itself that provides for higher DBI in general comparisons, but rather the surrounding income which allows for a more diverse and iron bioavailable diet.

The secondary aim of this analysis was to determine which foods, within any diet, could most effectively and efficiently improve DBI intakes, through either increasing iron or iron enhancing factors, among vegetarian diets. In general, these findings show that dietary improvements for DBI intake vary in effect based on diet type, however some foods consistently improve DBI intake more than other food groups, including green leafy vegetables and other vegetables. An additional 200 mg of GLVs to any cereal-based adult diet will increase DBI intake sufficiently to supply 50% of individual iron requirements. Achieving such a high intake of GLV, and the assumption that the rest of the diet would not change as a result, may not be a realistic assumption. However, a sense of the relative impact of GLVs on DBI is cause for more research and may warrant an efficacy trial in the vegetarian context. Results on the effect of pulses reveal that they are not the most effective provider of bioavailable iron across any diet type with the exception of rice diets, but their impact is mild. Again, an efficacy trial among vegetarian subjects would be needed to confirm these results.

Internal Validity The limitations and threats to internal validity with regards to the dataset and application of the algorithm are the same in this analysis as they were for the analyses in Chapter 2 (see Discussion section). As mentioned previously, the lack of data by mealtimes is a limitation for this analysis as the algorithm is designed to analyze dietary bioavailability for each individual meal. Also, the lack of data on iron status for each individual greatly reduced the intra-person variability around DBI intakes, as all women and all men had to be given an estimated serum ferritin value for the algorithm calculation. However, having accurate serum ferritin measures may not have solved our problems with determining iron absorption rates, since serum ferritin is highly elevated in cases of infection. This should be considered in the further refinement of iron bioavailability algorithms for use in developing country context, where infection rates tend to be high. Estimates for serum ferritin allowed us to circumvent this problem, however it was not ideal. An ideal measure for iron status is still needed in the field of nutrition.

Sensitivity analysis for the iron content of rice was conducted to determine if our findings are sensitive to our assumption of 0.7 mg/100 g of iron in rice, since most data on rice iron content is closer to 0.3 mg/100g. Results from the regression of cereal-based diets on DBI intake show that the lower rice content produces minor shifts in odds ratios and no changes in significance (except for sorghum diets where the odds ration drops from 0.73 to 0.50 and becomes significantly more protective than rice diets ($p<0.001$)). In general all cereals, except maize, become even more protective as the cereal-base when compared to rice diets which is the direction we would assume. The effect of time and state variables remain unaltered. These results are not surprising as overall iron content of rice is so low and there is no effect on bioavailability, there is simply a shift in the relative difference between rice diets and each of the other diets. Sensitivity analysis for the sub-analysis on poorest tertile women also only shifts the starting point of DBI intake down among rice consumers, but does not change the 'slope' or rate of improvement for any individual dietary modification.

Explanation of mechanisms The findings on the effect of additional pulse consumption on DBI across various cereal-based diets are both concerning and interesting. These are interesting findings as it was theorized that the decline in pulses over the course of the green revolution might have put people at *greater* risk of iron deficiency. Absorption of iron from pulses are known to be quite low (0.84% - 1.91%) [96] but our analysis indicate that within a certain range of consumption the iron contribution of pulses may be more than offset by its phytate content. Further analysis on the affect of pulses on bioavailability should be conducted before any conclusions can be made, however, as pulses are purported to be a good vehicle for iron, both as an un-enhanced and as an iron-biofortified crop. However, even if the findings were replicated in an *in vivo* study, any recommendations should not attempt to limit consumption of pulses to the diet. This is because of the very important contribution pulses provide for protein (particularly the amino acid lysine which is generally limiting in cereals), folate and fiber intakes. Work on biofortified beans may best be improved through a reduction in phytate and tannin content as much as the enhancement of iron.

As an indicator of iron deficiency anemia, our ‘Low DBI’ measure of DBI<50% of basal requirements is moderately useful, but could be improved. Part of the low negative predictive value of our indicator is that there are other causes of anemia in this population (e.g. vitamin A and B12 deficiency, thalessemias, and malaria). Another explanation is that there is a protective effect of low iron stores on absorption of iron. Whereas the algorithm cannot adjust for low stores on an individual level, biological reality is that individuals who experience chronic iron deficiency will have enhanced uptake of iron, therefore not creating a linear relationship between low iron intakes and risk of iron deficiency anemia. Unfortunately we do not have data on iron status, which presumably would better test our indicator, as we are only trying to estimate iron deficiency anemia and not anemia in this population. Given the day-to-day variation in dietary intake, we can expect one-day bioavailable iron intake to not be very sensitive to general anemia, but may provide a good screening tool to identify high risk of iron deficiency-caused anemia. If serum ferritin values were available the test of this indicator could be completed. This would be a valuable contribution as serum ferritin is an expensive test. Likely more than one 24-hour recall per individual would be required to test this well.

Policy implications As seen in Chapter 2, the use of the bioavailability algorithm in conducting this work was crucial, especially in this work which analyzed cereal-specific bioavailabilities. Cleary the ICMR estimates would not have been useful as we were partially testing them. If we had compared cereal-based diets purely on their ability to provide iron alone we would have ended up with very different conclusions. Using the bioavailability algorithm provided an additional level of precision in determining difference among cereals. Analysis indicates that finger millet and pearl millet diets, though generalized in ICMR estimates, yield different bioavailability rates. Assessing bioavailability through the ICMR estimates would have created a large nondifferential contribution to the error in comparing across individuals within diets and we would not have been able to analyze individual foods' impacts on DBI.

Future Research Biological research using tracer iron isotopes would be warranted before further conclusions can be made about the role pearl millet, GLVs and pulses might play in combating iron deficiency. A key finding in the analysis is that not all millets should be categorized together in nutritional education or in nutritional evaluation due to their very different nutrient content. There are many types of millets available in India for consumption but *ragi*, finger millet, tends to cost as much as *bajra*, pearl millet, at least in examining their government minimum support prices. Pearl millet stands above the rest in providing for DBI across any income group. Also, more needs to be understood about how finger millet is prepared, some may malt or ferment the finger millet which would degrade a lot of the phytates and produce ascorbic acid, greatly improving its bioavailability. There are potential implications for Africa in these millet diets. Finger millet is an African millet brought over to India many years ago, and its consumption is far more predominant in Africa. Further examination of finger millet and risk of iron deficiency should be conducted in the context of the African diet.

The promise of pearl millet in combating iron deficiency must also be tested further, first through *in vivo* studies and then through human trials. As seen in Chapter 2 in Gujarat in 2000-01 the surge in pearl millet consumption brought on by a drought and/or an earthquake served to protect consumers from lower DBI intakes. Data from

Gujarat may be further analyzed, using a quasi-experimental design, to determine if pearl millet consumers were indeed responding to food insecurity. Quantitative research can help determine perceptions of pearl millet among consumers. Pearl millet is consumed by both rural wealthy and poor in Gujarat and therefore not considered an 'inferior good' therefore its promise would not be limited by social acceptability in regions where it is grown, though likely outside Gujarat or in urban areas some social marketing would be necessary should it prove to be an effective way to improve DBI intakes in *in vivo* studies.

As seen in the diet of Orissans, green leafy vegetable consumption can greatly enhance iron absorption. Orissans consume about four times as much GLVs as individuals from any other state examined and therefore show a significant improvement in their bioavailability rate (7.3%), higher than the estimated rice-bioavailability (5%) and as close to that of meat-consuming Keralans (11%) than other vegetarian rice-consuming states, like Tamil Nadu (4%). The promotion of GLVs in the diet may require social marketing campaigns as GLVs tend to be consumed among the poorest populations, and therefore are considered inferior or 'hunger foods.' The increasing trend in vegetable production has undoubtedly contributed to the bioavailability improvements seen. Agricultural programs should continue to promote vegetable production, and think of subsidizing vegetable purchase among the PDS-targeted poor population, through programs similar to food stamp programs in the US.

India's burden of iron deficiency anemia is great and will require much political and social effort to combat effectively. Dietary behaviors can be difficult to change but as seen by dietary changes examined in Chapter 2, availability and affordability offer a large impetus to consumers to change consumption. Promotion of pearl millet and GLV consumption can be achieved through current public distribution mechanisms and educational campaigns that target the poor and vulnerable in India.

CHAPTER 4

IRON BIOFORTIFIED CROPS: THEIR POTENTIAL IMPACT IN IMPROVING DIETARY BIOAVAILABLE IRON IN INDIA

Introduction

Iron deficiency in India is pervasive and persistent, with anemia prevalence rates close to 70% for children and 60% for women. All areas of India are affected, rural and urban, poor and wealthy. A heavy reliance on cereals for iron intakes and the predominance of the vegetarian diets makes most Indian consumers vulnerable. Additionally, iron supplementation programs in India have been unsuccessful in reaching their targeted audience. Biofortification of major Indian cereals for higher iron content has been discussed as a viable way to reach millions of consumers in India, especially the rural population where health resources and dietary alternatives are limited.

Background

Iron deficiency anemia in India

With iron deficiency anemia rates over 70% of women and children [1, 2, 5, 7, 10], India is host to the largest population of anemic individuals in the world. The most recent Demographic Health Survey estimates for 2005 anemia prevalence among ever-married women, children, pregnant women and men are 56.2%, 79.2%, 57.9%, and 24.2% respectively [14]. This high prevalence of iron deficiency in India has been attributed to both low iron intakes and low iron bioavailability from diets with high levels of cereal consumption and low intakes of animal source foods [15].

The Indian states of Kerala, Gujarat, Andhra Pradesh, Karnataka, Madhya Pradesh and Uttar Pradesh show increased anemia prevalence in all three sub-populations over the past 6 years. These reports have unleashed substantial political concern in India about the country's failure to reduce anemia prevalence [35, 36].

Unsuccessful anemia prevention program

Although iron folate tablets have been distributed for over 30 years in India to pregnant and lactating women as well as children under the age of five, evaluations show that no biological effect has been seen on the targeted population. Less than 20% of women and less than 1% of children were reported to have been given the supplements [37, 38]. Due to the prohibitive cost of supplementation and fortification programs in a country as large and diverse as India, micronutrient intakes are still highly dependent on basic food crops, and will remain that way for a long time to come for the most inaccessible rural populations.

Dietary iron

Most dietary iron in India comes from cereals due to its heavy consumption in a predominantly vegetarian population [55]. Data from ICRISAT Village Level Survey on dietary intakes from rural Maharashtra and Andhra Pradesh indicate that between

73% - 82% of iron intake comes from cereals [56]. The cereals most commonly consumed in India are rice, wheat, and the coarse or traditional cereals of sorghum and millets. Both sorghum and millet contain roughly four times the concentration of iron than rice, gram for gram. See Appendix B for a full list of nutrient content for major Indian food items. Despite the iron content of traditional cereals, according to the National Nutrition Monitoring Bureau (NNMB) in rural areas in 1997 only 5.6% of household surveyed were consuming at or above the recommended intake for iron (28 mg/CU/day) [49].

Iron-biofortified rice and wheat

Recent efforts by HarvestPlus, co-led by the International Food Policy Research Institute (IFPRI) and the International Center for Tropical Agriculture (CIAT), have aimed to develop biofortified staple food crops [97]. These crops are bred to have higher micronutrient content in an effort to alleviate micronutrient deficiencies in the poorest countries, and can have the greatest impact among agricultural-based populations far removed from markets and heavily reliant on staple crops in their diets. A recent report by Stein [98] indicates that the introduction of iron-biofortified rice and wheat in India can reduce the burden of iron deficiency anemia, as measured through disability-adjusted life years (DALYs), by 19%. In rice consuming areas this burden can be relieved by as much as 29%. The study does adjust for low iron bioavailability in its estimates, based on the ICMR estimated levels. However individual-level bioavailability variation in iron bioavailability across diets as well as the potential impact on combating iron deficiency anemia was not estimated.

Biofortification efforts provide a viable strategy to improve iron intakes in India. Efficacy trials of iron-biofortified rice among healthy religious sisters in the Philippines have been shown to increase iron intakes and total body iron stories in women by 20% and cut in half their probability of consuming insufficient dietary iron [99]. Unlike supplementation and fortification programs, biofortified foods have the potential to reach rural poor families easily since they do not require a centralized mill or market.

Iron bioavailability

Major cereals in India offer varying amounts of iron, and iron content is generally related to the content of iron inhibiting compounds in the cereal. See Appendix B for the abbreviated food composition table used in India. Therefore the percent of iron that is bioavailable to humans varies depending on the major cereal in the diet, as well as the surrounding diet. Since only between 1% and 25% of iron is able to be absorbed by the body, iron bioavailability is crucial to determining risk of iron deficiency. A diet high in iron but low in bioavailability may provide less iron than a diet low in iron but high in iron bioavailability. The Indian Council of Medical Research estimates iron bioavailability rates for rice, wheat/millet and mixed cereal diets to be in the ratio of 5% : 2% : 3% with individuals of lower iron status having higher ratios (given the upregulation of absorption in deficiency individuals)[19]. However, these bioavailability rates are limited in their ability to allow for variation due to dietary quality within each cereal-based diet. For example, an individual consuming a millet-based diet who also consumes a lot of vitamin C (an enhancer of iron absorption) may absorb iron at a higher rate than an average rice consumer.

Individual variation in bioavailability can be estimated using an iron bioavailability algorithm. These algorithms calculate rates of absorption based on various factors including the inhibitors and enhancers of iron in the diet, the total iron consumed and the iron status of the individual [57, 67, 68, 70-72].

Objective

The objective of this paper is to quantify the potential impact of iron-biofortified rice and wheat in India on intake of dietary bioavailable iron and therefore reduction in the risk of iron deficiency in the population.

Methods

The primary outcome measures in this analysis were average improvements in total iron, dietary bioavailable iron (DBI) and the percent of the population shifting out of risk of iron deficiency anemia. Secondary data analysis was conducted on 24-hour recall data provided by the National Nutrition Monitoring Board (NNMB). These data were collected in rural areas in 2005, covering seven states of India including Kerala, Tamil Nadu, Karnataka, Andhra Pradesh, Maharashtra, Gujarat and Orissa. The NNMB has been collecting high quality dietary data in India since 1975. All enumerators are nutritionists or doctors and are trained in dietary data collection methods. See Methods section in Chapter 1 for a more complete explanation of this dataset.

Analyses include all individuals from 2005 dietary surveys including non-adults over 3 years of age and including pregnant women (n=22,221). Inclusion for all subgroups made for the most reliable estimates on shifts in IDA risk, given the variation within subgroups for iron intakes and for iron requirements. Only dietary surveys from 2005 were included to best approximate current dietary patterns. Children 1-3 years of age were excluded from all analysis given that many within this subgroup were breastfeeding, potentially complicating results that were focused on dietary change. It is noted that dietary records for young children can be difficult to construct.

Improved iron content values for biofortified rice closely approximate baseline values found in the Indian Food Composition Table. According to current CGIAR information [62], nutrient target levels for iron-biofortified rice are 0.8 mg/100g of raw rice, whereas Indian FCT values are 0.7 mg/100g in raw milled rice. Therefore iron content estimates for rice in India are much higher than those used by HarvestPlus/CIAT at 0.3 mg/100 g of raw rice. In order to be consistent with nutritional findings in India, FCT values will be used but we base our projected final iron content on the **percent improvement** in iron not on the final iron content expected. We used their ‘pessimistic scenario’ of only a 100% improvement in iron content in rice, given the already high levels assumed, and used their ‘optimistic scenario’ of a 60% improvement in iron content in wheat, as seen in **Table 4.1**.

Food composition data for India were most recently updated in 1989, and were gathered from laboratory analyses conducted in various Indian universities and/or

Table 4.1. Assumptions made about iron improvements in rice and wheat

	HarvestPlus targets (iron content per 100g)	Percent Improvement	Indian FCT value (baseline iron content)	Percent improvement tested
Rice	0.3 mg to 0.8 mg	166%	0.7 mg	100%
Wheat	3.8 to 6.1 mg	60%	4.9 mg	60%

from published data from Indian foods using AOAC-approved analyses for mineral content [55].

We assumed that only iron content values change in these two biofortified crops, and not the content of the iron enhancers or inhibitors. In determining bioavailable iron, an individual's bioavailability rate determined from the algorithm was applied to their new iron intake values. The Hallberg & Hulthen algorithm was applied to the database to calculate individual dietary bioavailable iron intakes. Serum ferritin adjustments were made to reflect low iron status for all women and children and just below normal status for men. This is due to the assumed iron deficiency prevalence in the population. For more information on how the algorithm was used, please see the Methods section in Chapter 2.

Statistical Analyses All statistical analyses were completed using Stata 9.2 by StataCorp [81]. A breakdown in the effect of biofortification for different age groups, socioeconomic strata and states is presented. For all pooled analysis complex survey design adjustments were made to ensure representative proportions to the total 7-state population. Risk of iron deficiency was determined from previous indicators as DBI<50% of mean basal requirements for age, sex and physiological status.

Calculations for the shift in the population no longer at risk of iron deficiency were conducted using contingency tables, where the proportion of individuals no longer at risk but once at risk over the total sample is used as the percent of the total population, as shown in **Table 4.2**. Because we were controlling for design effect, statistical calculations provide the weighted proportion in the B cell directly, and not as the number of observations falling in that cell.

Table 4.2: Example contingency table for calculating shifting population

		Without Biofortification	
		No risk	Risk
With Biofortification	No Risk	A	B
	Risk	C	D

Calculation of the proportion of the population which would shift out of risk of iron deficiency = B/(A+B+C+D) (where C=0).

Results

Respectively, rice and wheat provide 13.4% and 15.3% of the total iron in the average rural Indian diet across these seven states (1.79 g/person/day and 2.04 g/person/day), but rice provides 16.4% of the total DBI whereas wheat is only 10.9%, due to the lower bioavailability of iron in wheat.

As seen in **Table 4.3**, replacing current iron and wheat with biofortified varieties would improve the average amount of bioavailable iron in the population by 23% from 0.55 mg to 0.67 mg per day. In this area of the country, South India especially, iron-rich rice would improve DBI more than iron-rich wheat. Biofortified rice would provide an additional 1.79 mg/day of rice to the average consumer, which is similar to findings from experimental studies in the Philippines that found a 1.41 mg/day gain in iron from rice among religious sisters [99].

Across age groups the percent improvement is consistent, i.e. there are no age groups that would benefit more than another. Biofortification would improve DBI among the poorest tertile by 25% and by 20% among the richest. This disproportionate aiding of the poor is important as biofortification programs aim to target the most vulnerable populations, while still improving the foundation of iron intakes across the population. Among the states, the rice-consuming states of Tamil Nadu, Andhra Pradesh and Orissa would see the greatest improvement in DBI (32% - 35%) due to the high consumption of rice in those states (342 g, 408 g and 419 g on average in 2005 across this sample, respectively). Karnataka, consuming rice mixed diets, would see modest gains (a 19% improvement in DBI) while Maharashtra and Gujarat, consuming more wheat than rice would gain ~10% additional DBI, due more to the wheat than rice.

The effect of these improvements on the underlying distribution of iron requirements

Table 4.3. The impact of iron-biofortified rice and wheat on iron intake and iron deficiency risk

Projected shift out of risk of iron deficiency in seven rural states of India

	Current intake in whole diet		Additional intakes			Population shifting out of IDA risk[3,4]	
	Iron mg/day	DBI mg/day	Iron mg/day	DBI mg/day	Improvement %	%	Million people
Biofortified Rice[1] only	13.4	0.55	1.79	0.09	20.3	3.6	10.18
Biofortified Wheat[2] only	13.4	0.55	0.41	0.01	2.4	0.6	1.70
Both biofortified	13.4	0.55	2.20	0.12	22.7	4.2	11.87
By income:							
Richest tertile	15.2	0.64	2.1	0.11	19.5	3.7	3.49
Poorest tertile	12.1	0.48	2.2	0.10	24.6	4.4	4.15
By age:							
Adults (>=18)	14.7	0.61	2.4	0.12	23.1	3.8	-
16-17 yr olds	14.0	0.61	2.1	0.12	22.2	3.8	-
13-15 yr olds	12.3	0.53	2.0	0.11	23.1	3.8	-
10-12 yr olds	10.9	0.42	1.7	0.08	22.6	3.8	-
7-9 yr olds	9.0	0.33	1.5	0.06	23.2	5.3	-
4-6 yr olds	7.6	0.32	1.2	0.05	22.0	7.2	-
By state:							
Kerala	10.1	1.29	2.2	0.24	23.2	5.6	1.20
Tamil Nadu	8.9	0.38	2.6	0.10	31.6	6.6	2.43
Karnataka	13.7	0.24	1.9	0.04	19.0	1.6	0.50
Andhra Pradesh	9.5	0.45	3.0	0.13	34.8	3.9	2.31
Maharashtra	16.9	0.39	1.5	0.04	11.4	1.7	0.82
Gujarat	23.5	0.65	1.2	0.03	6.5	1.6	0.55
Orissa	10.3	0.88	3.0	0.23	33.4	10.3	2.82

[1]biofortified rice assumes 100% improvement (0.7 mg/100g to 1.4 mg/100g)

[2]biofortified wheat assumes 60% improvement (4.9 mg/100g to 7.8 mg/100g)

[3]risk defined as DBI <50% of basal requirements by age, sex and physiological status

[4]rural population estimates extrapolated from 2001 census data (www.censusindia.gov accessed 11/15/2007), total rural population in these seven states in 2005 (282.7 million people)

tells us what percent of the population would shift out of risk of iron deficiency anemia (IDA). Replacing current rice and wheat varieties to iron-biofortified varieties would shift roughly 4% of the total population out of risk, this is equivalent to 10.9 million individuals in rural areas of these seven states. Again, the poorest third of the population would benefit more than the richest third seeing a larger shift in the population moving out of risk (4.4% versus 3.7%). These improvements are large on a relative scale. There are few iron interventions that can boast improvements impacting millions. However, it must be kept in mind that the total rural population of this area is 283 million and currently roughly 72.6% (205 million people) are currently considered to be at risk of IDA, from our cut-off estimate. These improvements alone would still leave the vast majority of the population at risk

Because Indian food composition tables report that milled rice contains more than twice the amount of iron (0.7 mg of iron per 100 grams of rice) as most biofortification studies report to be baseline content (0.3 mg of iron per 100 grams of rice) [62, 63], a sensitivity analysis of the iron content we used is tested against the other value. Results indicate that if baseline rice in India contain 0.3 mg of iron per 100 grams of rice, and biofortification efforts double iron content or triple iron content, large impacts can be still be seen (see **Table 4.4**). Even under pessimistic scenarios (100% improvement in iron content at baseline 0.3 mg/100g) roughly 4.5 million people could shift out of risk of IDA, and 9.0 million if iron content is tripled.

Discussion

The purpose of this work was to quantify the impact iron-biofortified crops would

Table 4.4. Sensitivity analysis results

The impact of biofortified rice and wheat on iron deficiency risk at different iron content baseline values for rice

Baseline and projected mg of iron in 100g rice	Current intake in whole diet		Additional intakes			Population shifting out of risk[2,3]	
	Iron	DBI	Iron	DBI	Improvement	%	Million people
Different Assumptions[1]:	mg/day	mg/day	mg/day	mg/day	%		
0.7 to 1.4 (100%)	13.4	0.55	2.2	0.12	22.7	4.2	11.9
0.3 to 0.9 (200%)	12.4	0.50	1.53	0.08	22.1	3.2	9.0
0.3 to 0.6 (100%)	12.4	0.50	0.76	0.04	11.1	1.6	4.5

[1]biofortified wheat is included in all three analyses and assumes 60% improvement (4.9 mg/100g to 7.8 mg/100g)
[2]risk defined as DBI <50% of basal requirements by age, sex and physiological status
[3]rural population estimates extrapolated from 2001 census data (www.censusindia.gov accessed 11/15/2007), total rural population in these seven states in 2005 (282.7 million people)

have on improving not just intakes of iron, but also bioavailable iron, for rural consumers in India. The biofortification of staple crops for improved iron could yield substantial improvements in iron intakes for many Indians, particularly in rice-consuming states. Replacing current rice and wheat varieties with iron-biofortified varieties would boost the baseline DBI intakes across the population and shift over 11 million people out of risk for iron deficiency anemia, according to our estimates. Findings represent fairly conservative estimates for iron improvement given that some iron rice improvement programs are also using genetic modification to increase iron bioavailability within the grain [100, 101] which warrant separate examination of their potential impact.

Internal Validity A full review of the limitations to the application of the bioavailability algorithm on the Indian dietary dataset can be seen in more detail in Chapter 2 under the Discussion section. In summary, the key limitations to the dataset were the lack of data on individual mealtimes and on individual iron status (serum ferritin was used in the algorithm). The Hallberg & Hulthen algorithm was designed to estimate bioavailabilities from individual mealtimes but data were presented as items and quantities consumed within the last 24-hour period. The impact of volume on the algorithm calculation was found to be negligible (see Chapter 2) but the separation of food items and their impact on mealtime bioavailability was not captured. Having iron status reduced individual variability around iron absorption and reduced the precision of our estimates. However, there are problems inherent in using serum ferritin as a measure of iron status as it can mask iron deficiency when infection is present. Estimating serum ferritin based on sex and age helped circumvent this problem, but was less than ideal. Refinement of iron bioavailability algorithms to include a better measure of iron status is recommended, though that measure is still not yet agreed upon by the nutrition community. The sensitivity analysis conducted on iron content of rice tells us that significant improvements in DBI intake would be seen even if the baseline content of iron in rice is less than half the assumed content.

The disproportionate gains in DBI seen by some states, like Kerala, can be explained through a distribution graph. The IOM distribution for iron intakes and their estimated average requirements is an S-curve [60]. In Appendix D we have adapted it to DBI intakes and requirements using the bioavailability rates set forth by the ICMR for adult non-pregnant, non-lactating women (using 5.5% for mixed cereal diets). Here we see why DBI intakes see the greatest increase in percentile or requirement met closer to the estimated average requirement (EAR). On the other hand, near the 2.5%ile and 95.5%ile we see small improvements in requirements met per additional DBI consumed. This is why a state like Kerala, although showing a smaller improvement in DBI intakes (23%) compared to Andhra Pradesh (35%), would shift 5.6% of its population out of risk, when Andhra Pradesh would only shift 3.9% of its population. The bulk of Kerala's population sits further up the iron requirements distribution, with more people approaching the EAR (estimated average requirement) than populations like Andhra Pradesh, and therefore small increments shift more people over the cut-off. Our cut-off value was established at DBI <50% of recommended basal requirement (the EAR itself) because using the EAR we found almost the entire population to be deficient in dietary iron, which is consistent with estimates of biological iron deficiency (99.9% of the population of women and children are iron deficient based on extrapolation of the prevalence of anemia). Using DBI<50% of the EAR (which at 0.75 mg/day is roughly 2.5th%ile on a standardized curve for basal requirements) as the cut-off, a better estimate of those at risk of iron deficiency anemia is achieved. Also, with anemia rates between 50% and 80% this was a realistic cut-off value. As we saw from our results in Chapter 2, roughly 80% of the population of women was at risk of low DBI (dietary iron deficiency).

External Validity Due to the nature of the dataset available, the full potential impact on iron-biofortified wheat and rice in all of India was not possible. Extrapolation of findings from the seven states analyzed to the rest of India should be cautioned. This is especially true for the results for iron-biofortified wheat, which in this sample of states is only a secondary cereal not a primary cereal. Wheat was included in analysis to see the potential impact among secondary consumers of wheat. A full analysis in northern states would be warranted if valid and recent dietary data could be found.

Policy implications & future research The introduction of iron-biofortified rice is certainly worthy of discussion among policy makers in India. Results from this work contributes to the mounting evidence that iron-biofortified crops can shift the base of the population closer to sufficiency, although in and of itself biofortification is not enough. Supplementation programs need to be more effectively administered and fortification of flours should be decentralized so rural consumers gain access.

Orissa, Andhra Pradesh and Tamil Nadu would most benefit from introducing biofortified rice to their food supply and should encourage state agricultural research centers to breed varieties adapted to local conditions. Although adoption of biofortified rice by farmers may take years to implement, higher minimum support prices for these varieties would encourage adoption. In addition, demand creation could begin through education campaigns and by introducing biofortified varieties in the already-established public distribution system (PDS). An added benefit is that the PDS system already appropriately targets families in need. Further analysis on the potential impact of biofortified crops in India would be valuable for setting both nutritional and agricultural research priorities. Biofortified varieties should not require more inputs or result in lower yields than currently used varieties or farmer adoption will be minimal, even if the seed is subsidized and the support prices are higher.

It should be stressed again that the biofortification approach, while having the potential to reach many individuals, is not sufficient in and of itself to significantly reduce iron deficiency in rural India. Although 12 million people could shift out of risk of iron deficiency, 270 million still remain iron deficient in rural areas of these seven states. Supplementation and fortifications programs as well as other food-based approaches should continue efforts to target highly vulnerable subsets of the population. The advantage of the biofortification approach is its wide and deep reach to help elevate iron levels for the whole population, and not just for those in urban, more accessible areas.

CHAPTER 5

CONCLUSIONS

India has amongst the highest rates of iron deficiency anemia in the world, and nearly every woman and child in the country can be considered iron deficient. According to WHO, the high prevalence of anemia in India is classified as a problem of "severe public health significance." Few gains have been made in preventing or reducing iron deficiency in India, perhaps because it has one of the highest dependencies on plant foods for the diet in the world [85]. Fortification and supplementation programs are terribly inadequate in providing to the millions of women and children even in urban areas, much less rural areas of India. Improving iron intakes through the diet will have the greatest and most sustainable impact on alleviating iron deficiency anemia.

This research sheds some light on historic trends in iron intakes, the connection between iron nutrition and cereal consumption, and the potential promise of some Indian foods for improving bioavailable iron to reduce the risk of iron deficiency for one of the world's most nutritionally vulnerable populations. Evidence indicates that dietary changes in these seven rural states of India from 1975 to 2005, whether due to agricultural, socioeconomic or a combination of shifts, resulted in improved dietary bioavailable iron and therefore reduced the risk of iron deficiency anemia in the adult population. It is unclear whether risk of iron deficiency would have been worse had the green revolution not occurred as we do not know what would have happened in its place. Yet iron deficiency anemia continues to be a major problem in India despite gains in food security in India since the early 1970s with the progress of the green revolution. Reasons include the heavily reliance on cereal crops in the Indian diet which result in very low iron bioavailability. However in most of India, cereals also provide the bulk of iron to the diet. Although agricultural gains of the 1970s and 1980s improved DBI intakes in rice-consuming Indian states, as was seen in Chapter 2, results from Chapter 3 indicate that rising incomes had more to do with the improvements than rice itself. Rice ranked near the bottom of the list of cereals in providing sufficient DBI when income was controlled for in analysis.

Short-term recommendations to improve bioavailable iron in the Indian diet should be geared towards the promotion of vitamin C-rich foods in the diet during mealtimes, ideally through the consumption of green leafy vegetables. Further reduction of tannins and phytates in the diet may not be the best solution as they also have positive health effects in the diet as antioxidants (tannins) and for their association with dietary fiber (phytates). Long-term recommendations to improve bioavailable iron in the Indian diet should also incorporate efforts to improve overall iron intakes, the promotion of iron biofortified rice would help boost iron intakes, albeit modestly. Multiple approaches will need to be used to make long and lasting change in the diet for the average rural Indian, who can be inaccessible to the market and dependent on local agriculture for their dietary needs. Approaches that consider the whole food supply are crucial when looking at bioavailability and nutrition in times for those most vulnerable.

When the world’s poor rely on staples for 70% of their caloric needs, a wider array of affordable and nutrient-rich staples may more effectively combat micronutrient malnutrition than any other intervention. Without this diverse foundation of basic foods the rural poor, who are often missed or untouched by nutrition programs, will continue to suffer from overt malnutrition and disease at disproportionate rates.

Another important contribution of this research was the application of a bioavailability algorithm to dietary intake data in a developing country context. Such algorithms are important tools in the field of nutrition research and need to be further tested and refined to be applicable and useful in resource-poor settings where isotopic testing of iron absorption is not a viable option. The Hallberg & Hulthen algorithm used in this research proved to be consistent and reliable, with estimates mildly underestimating bioavailability rates. However, the sensitivity of the algorithm to adjustments in the diet prove them to be far more useful than bioavailability estimates used by the ICMR based on the cereal-base of the diet. This is especially true given the large variability within rice-based diets (Keralan fish diets vs. Andhra Pradesh vegetarian diets), and the large disparity found in bioavailability rates among the millets, despite their being grouped together in ICMR estimates.

In a wider context, food choices are heavily dependent on food availability and cost. Agricultural growth is often measured as improvements in yield, and therefore caloric production, whereas the provision of nutrients is not deliberate. It is important to establish if caution should be advised when setting food production priorities based on calories alone. The success of improved food production over the past thirty years in India is without question, but there is still room for improvement with respect to the provision of adequate nutritious food for all in India. Lessons learned from India can help every country tap their nutritional and thus human potential more wisely. Finally, sub-Saharan Africa is still waiting for an agricultural revolution to solve its problems of hunger and can build on India's progress.

APPENDIX A

Map of India

APPENDIX B

Indian Food Composition Table

In milligrams per hundred grams of raw food

FOODNAME	Calcium	Vitamin C	Total Iron	Non-Heme Iron	Heme Iron[1]	Tannin[2]	Phytic Acid
BAJRA (Pearl millet)	42.00	0.00	8.00	8.00	0.00	12.90	493.50
JOWAR (Sorghum)	25.00	0.00	4.10	4.10	0.00	77.00	602.00
MAIZE,dry	10.00	0.00	2.30	2.30	0.00	24.10	1071.00
RAGI (Finger millet)	344.00	0.00	3.90	3.90	0.00	360.00	731.50
RICE (milled)	10.00	0.00	0.70	0.70	0.00	0.00	287.69
WHEAT FLOUR	45.00	0.00	4.90	4.90	0.00	22.66	795.38
OTHER CEREALS	31.00	0.00	9.70	9.70	0.00	44.72	0.00
BENGAL GRAM	71.08	0.00	6.26	6.26	0.00	38.48	496.64
BLACK GRAM	154.00	0.00	3.80	3.80	0.00	24.84	591.50
GREEN GRAM	86.89	0.00	4.02	4.02	0.00	119.48	679.71
KHESARI, dhal	90.00	0.00	6.30	6.30	0.00	0.00	378.00
LENTIL	69.00	0.00	7.58	7.58	0.00	71.70	350.00
RED GRAM	72.84	0.00	2.68	2.68	0.00	49.68	595.00
SOYABEAN	240.00	0.00	10.40	10.40	0.00	45.00	980.00
OTHER PULSES	207.00	0.00	7.40	7.40	0.00	192.24	490.36
GREEN LEAFY VEGETABLES	244.00	63.00	5.20	5.20	0.00	180.29	69.94
OTHER VEGETABLES	36.00	29.00	1.40	1.40	0.00	25.65	32.66
CARROT	80.00	3.00	1.03	1.03	0.00	13.00	14.00
ONION	47.00	11.00	0.60	0.60	0.00	6.00	0.00
POTATO	10.00	17.00	0.48	0.48	0.00	0.00	49.00
TAPIOCA	50.00	25.00	0.90	0.90	0.00	0.00	31.15
OTHER TUBERS & ROOTS	35.00	12.00	1.00	1.00	0.00	0.00	19.89
CASHEWNUT	50.00	0.00	5.81	5.81	0.00	0.00	1866.00
COCONUT,dry	400.00	7.00	7.80	7.80	0.00	0.00	0.00
COCONUT, fresh	10.00	1.00	1.70	1.70	0.00	0.00	0.00
GROUNDNUT CAKE (pressed, with some oil removed)	213.00	0.00	2.80	2.80	0.00	0.00	1760.00
OTHER NUTS & SEEDS	213.00	0.00	8.00	8.00	0.00	356.85	1546.92
SPICES & CONDIMENTS	342.00	15.00	11.20	11.20	0.00	1113.41	206.47
AMLA (Indian gooseberry)	50.00	600.00	1.20	1.20	0.00	280.00	0.00
APPLE	10.00	1.00	0.66	0.66	0.00	160.00	0.00
BANANA RIPE	17.00	7.00	0.36	0.36	0.00	40.00	14.00
LIME&ORANGE	58.00	46.00	0.33	0.33	0.00	0.00	0.00
MANGO RIPE	14.00	16.00	1.30	1.30	0.00	0.00	20.00
MELON, sweeter	11.00	1.00	7.90	7.90	0.00	0.00	2.10
PAPAYA, ripe	17.00	57.00	0.50	0.50	0.00	0.00	14.00
TOMATO RIPE	48.00	27.00	0.64	0.64	0.00	0.00	7.00
OTHER FRUITS	31.00	35.00	5.30	5.30	0.00	26.56	47.95

FISH, FRESH	400.00	5.54	2.10	1.47	0.63	0.00	0.00
FISH, DRIED	1735.00	0.00	24.30	17.01	7.29	0.00	0.00
PRAWN	323.00	0.00	5.30	3.71	1.59	0.00	0.00
MEAT	61.40	1.29	3.52	1.83	1.69	0.00	31.68
FOWL (CHICKEN)	25.00	0.00	0.60	0.36	0.24	0.00	0.00
LIVER GOAT	17.00	30.00	6.30	5.29	1.01	0.00	0.00
EGG, hen	60.00	0.00	2.10	2.10	0.00	0.00	0.00
MILK	161.06	1.74	0.21	0.21	0.00	0.00	0.00
SKIMMED MILK, liquid	120.00	1.00	0.20	0.20	0.00	0.00	0.00
CHEESE (Kheer)	790.00	0.00	2.10	2.10	0.00	0.00	0.00
BUTTER	0.00	0.00	0.00	0.00	0.00	0.00	0.00
GHEE	0.00	0.00	0.00	0.00	0.00	0.00	0.00
HYDROGENATED OIL (fortified) Vanaspati	0.00	0.00	0.00	0.00	0.00	0.00	0.00
COOKING OIL (Gnut,ging,palm,must,coconut)	0.00	0.00	0.00	0.00	0.00	0.00	0.00
BETEL LEAVES	230.00	5.00	10.60	10.60	0.00	0.00	0.00
BISCUITS (salt)	120.00	0.00	0.50	0.50	0.00	0.00	180.00
BISCUITS (sweet)	120.00	0.00	0.50	0.50	0.00	0.00	116.00
WHEAT, bread, (white)	11.00	0.00	1.10	1.10	0.00	0.00	0.00
SUGAR CANE	12.00	0.00	0.16	0.16	0.00	0.00	0.00
JAGGERY	53.09	0.00	2.62	2.62	0.00	0.00	0.00
PAPAD	80.00	0.00	17.20	17.20	0.00	0.00	3.50
SAGO	10.00	0.00	1.30	1.30	0.00	0.00	3.50
TODDY	150.00	0.00	0.30	0.30	0.00	0.00	0.00
HORLICKS	272.00	0.00	1.30	1.30	0.00	0.00	0.00
FAREX	750.00	50.00	20.00	20.00	0.00	0.00	0.00
AMUL SPRAY	1000.00	35.00	0.60	0.60	0.00	0.00	0.00
MAHUA	45.00	40.00	0.23	0.23	0.00	0.00	0.00
COMMON SALT	0.00	0.00	0.00	0.00	0.00	0.00	0.00
OTHERS	0.00	0.00	0.00	0.00	0.00	0.00	0.00

All sources from National Institute of Nutrition [55] unless otherwise indicated

[1]data on the heme vs non-heme content of animal source foods were from Hallberg & Hulthen 2000 [57]

[2] data on tannin content are from the appendix in Hallberg & Hulthen 2000 [57] and/or from independent analyses [65, 78]

APPENDIX C

Hallberg & Hulthen Iron Bioavailability Algorithm

Individual dietary factor absorption ratios (AR):

PHYAR = $10^{(-0.30 \text{ x } \log(1 + \text{PHY}))}$

AAAR = 1 + 0.01 x AA + log (PHYP + 1) x 0.01 x $10^{\,0.8875 \text{ x } \log(\text{AA} + 1)}$

TAAR = (1 + 0.01 x MFP) x $10^{\,0.4515 - \{0.715 - 0.1825 \text{ x } \log(1+\text{AA})\} \text{ x } \log(1 + \text{TA})}$

CAAR = 0. 4081 + {0.5919/(1+ $10^{-\{2.022 - \log(\text{CA} + 1)\} \text{ x } 2.919\}}$)}

MFPAR = 1 + 0.00628 x MFP x (1 + 0.006 x PHYP)

SOYAR = 1 – (0.022 x soy)

EGGAR = 1- 0.27 x (number of eggs (grams egg/60g))

OHAR= 1.25 if any alcohol consumed

If TAAR >1 it was reset to =1, if EGGAR<0.2 set to 0.2, if SOYAR>0.56 set to 0.56.

Final calculations and adjustments:

NHARunadj = 22.1 x PHYPAR x AAAR x TAAR x CAAR x MFPAR x SOYAR x EGGAR x OHAR

HAR = CAAR x $10^{\,1.9897 - 0.3092 \text{ X } \log \text{SF}}$

DBI = ((NHAR x mg non-heme iron) + (HAR x mg heme iron)) x $(23/\text{SF})^{0.9409}$

Total absorption ratio (TAR) = DBI / total iron

Where:

PHYAR= phytate absorption ratio

PHY= phytate in the diet (in mg)

AAAR = ascorbic acid absorption ratio

AA= ascorbic acid in the diet (in mg)

TAAR = tannic acid absorption ratio

MFP= meat/fish/poultry in diet (in grams)

TA= tannic acid in the diet (in mg)

CAAR = calcium absorption ratio

CA= calcium in the diet (in mg)

MFPAR = meat/fish/poultry absorption ratio

SOYAR = soy absorption ratio

SOY= soy in the diet in grams

EGGAR = egg absorption ratio

OHAR= alcohol absorption ratio

NHARunadj= non-heme iron absorption ratio, unadjusted for iron status

HAR=heme iron absorption ratio, adjusted for iron status

SF = individual serum ferritin concentration in g/dL

DBI= dietary bioavailable iron, adjusted for iron status

TAR= total iron absorption ratio (includes heme and non-heme iron)

Source: Hallberg et al 2000 [57]

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